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DEVELOPMENT OF IMPROVED ANALYTICAL TECHNIQUES FOR DETERMINATION OF ASBESTOS IN WATER SAMPLES

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16. ABSTRACT
Sample preparation techniques were examined for the analysis of asbestos fiber concentrations in water. The carbon-coated Nuclepore technique using a polycarbonate filter proved to be superior to either the "drop" or the collapsed membrane filter technique. Compared with plasma ashing, ozone-ultraviolet light oxidation of water samples was found to be a simpler and superior technique for removal of organic materials. Experiments revealed that large proportions of the suspended asbestos fibers could become attached to the inside surfaces of sample containers. This effect was caused by trace organic materials of bacterial origin. Ozone oxidation, carried out inside the collection container, released the attached fibers into the water again. Initial experiments were carried out to determine the effectiveness of the attachment phenomenon as a fiber separation technique. Experiments into the nature of the scavenging effect of bacteria on container surfaces led to the development of stable reference dispersions of asbestos fibers. If bacteria and their products were excluded initially, and if absolute sterility was maintained thereafter, suspensions of both chrysotile and crocidolite appeared to be stable for long periods of time. Tests of reference suspensions in sealed glass ampoules stored for almost two years produced fiber concentration values statistically compatible with those obtained at the time of sample preparation.

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Analytical Chemistry Branch develops and assesses new techniques for identifying and measuring chemical constituents of water and soil.

A 3-year study was conducted to develop improvements in the analytical method for determination of asbestos fiber concentrations in water samples. The research produced an improved sample preparation and analysis methodology, a rapid screening technique to reduce analysis cost, and a new reference analytical method for asbestos in water. The analytical method for determining asbestos fibers in water is perceived as representing current state-of-the-art.

William T. Donaldson
Acting Director
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PREFACE

The Preliminary Interim Method for Determining Asbestos in Water was issued by the U.S. Environmental Protection Agency's Environmental Research Laboratory in Athens, Georgia. The method was based on filtration of the water sample through a sub-micrometer pore size membrane filter, followed by preparation of the filter for direct examination and counting of the fibers in a transmission electron microscope. Two alternative techniques were specified: one in which a cellulose ester filter was prepared by dissolution in a condensation washer; and another known as the carbon-coated Nuclepore^R technique which used a polycarbonate filter. In January 1980 the method was revised (EPA-600/4-80-005) to eliminate the condensation washer approach, and a suggested statistical treatment of the fiber count data was incorporated.

The research program described here is directed towards refinement of the revised interim method. It was recognized that clearly-defined methods for fiber identification were required, in addition to further standardization of the format for reporting of analytical results. Major improvements were also required in the method for removal of interfering organic materials. The use of ozone-ultraviolet light oxidation prior to filtration of the water sample was investigated and found to be superior to any other technique. This oxidation technique also eliminated the sometimes serious scavenging of fibers by sample container walls, an effect which had not been systematically investigated. Reference dispersions of asbestos fibers which are stable for long periods of time were also developed. These will now permit known analytical quality assurance samples to be submitted for analysis along with field samples. The lack of standard samples was one of the major limitations of asbestos fiber analyses.

The research program has allowed development of an analytical method which incorporates the best available technology. This method should be used when the most reliable analytical data are required.

ABSTRACT

A research program directed towards improvement of the analytical method for determination of asbestos fiber concentrations in water samples was carried out. Sample preparation techniques involving evaporation of small drops on transmission electron microscope (TEM) grids were found to yield samples with uneven deposits of particulate, and were thus unsuitable for quantitative determinations. The collapsed membrane filter technique was found to incur significant size-dependent fiber losses, which became more serious with increasing filter pore size.

Using naturally-occurring dispersions of both chrysotile and amphibole fibers, it was demonstrated that intra-laboratory analyses made by the same operator using the same instrument were statistically compatible at the 5% level.

Ozone-ultraviolet light oxidation of water samples was found to be a simpler and superior technique for removal of organic materials than plasma ashing. Experiments indicated that unknown and sometimes very large proportions of the suspended fibers may become attached to the inside surfaces of the container. It was found that this effect was a consequence of trace organic materials of bacterial origin, and that ozone-UV oxidation, carried out inside the collection container, released the attached fibers into the water again. Initial experiments were carried out to determine the effectiveness of the attachment phenomenon as a fiber separation technique.

The experiments into the nature of the scavenging of fibers by container surfaces led to the development of stable reference dispersions of asbestos fibers. It was found that if bacteria and their products were excluded initially, and if absolute sterility was maintained thereafter, suspensions of both chrysotile and crocidolite appeared to be stable for very long periods of time. Reference suspensions in sealed glass ampoules have now been stored for almost 2 years, and they still yield fiber concentration values statistically compatible with those originally obtained at the time of preparation.

A fiber classification system was developed which recognizes instrumental limitations, and if required, permits later re-evaluation of the raw data using different fiber identification criteria. A computer program was written which permits fiber identification on the basis of energy dispersive X-ray spectra and zone axis selected area electron diffraction patterns. The identification procedure operates by selection of minerals which are consistent with the measurements, using a library of data from 226 minerals. A standardized format for reporting of fiber counting data was also established, and a computer program written to facilitate this.

A reference analytical method was written (published separately), which

incorporates ozone-UV oxidation, fiber identification and classification, and a standardized reporting format.

A rapid screening technique was developed which can be used to eliminate from further study those water samples which have low fiber concentrations. This technique (published separately) is based on the alignment properties of asbestos fibers in magnetic fields.

This research program was performed under contract 68-03-2717 under sponsorship of the U.S. Environmental Protection Agency. This report covers a period from October 1978 to September 1981 and the work was completed as of September 1981.

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SECTION 1

INTRODUCTION

This research program had two primary objectives:

(a) to develop a rapid screening technique which can be used to eliminate from further study those water samples which have low fiber concentrations; and

(b) to develop the analytical procedure for determination of asbestos fiber concentrations in water to the point that a refereed method can be defined.

The rapid screening technique development was based on the alignment property of asbestos fibers in magnetic fields, and the results of these investigations are reported separately.[1]

Analytical techniques for determination of asbestos fiber concentrations in water samples have been generally based on transmission electron microscopy (TEM).[2] The existing procedure is essentially one in which each individual fiber detected in a known fraction of the sample is identified and measured. However, this existing procedure requires improvements in a number of areas.

(a) There must be a clearly-defined procedure for identification of fibers.

(b) It must incorporate a reliable means of oxidation of organic materials which interfere with the analysis.

(c) A standardized fiber counting procedure and reporting format must be defined.

(d) Reference suspensions must be incorporated so that quality assurance samples of known values can be introduced into analytical programs.

(e) It must be capable of yielding reproducible analyses when these are conducted by different laboratories starting from actual liquid samples of both naturally-occurring and laboratory-prepared fiber dispersions.

(f) There must be a demonstration of the precision and accuracy attainable, for both number and mass concentrations.

Since environmental water samples contain many particles of elongated shape which are not asbestos fibers, during TEM analysis it is necessary to examine each particle separately in order to discriminate the asbestos fibers. Where a fiber has limited electron scattering power its internal morphology may be visible in the TEM. This internal morphology in some circumstances may be sufficiently characteristic to be considered as an identification, but in general other information is required before a fiber can be confidently identified. In effect, the problem is one of determining the precise crystal structure and composition of an individual particle which has crystallized in a fibrous habit. If the analytical method is to be used in a regulatory sense, the fiber identification procedure must allow for no other interpretation to be made. Two fiber identification techniques are available on a modern analytical electron microscope: selected area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA). The SAED technique permits investigation of the crystallography of an individual fiber, and EDXA permits a qualitative, or, in some circumstances, a quantitative measurement of the chemical composition of a fiber.

The inadequacy of EDXA measurement alone has been reported.[3] The problem of fiber identification is illustrated in Figure 1, which shows the compositions of the more common fibrous silicate minerals which are collectively thought of as asbestos. It will be noted that within one type there can be a substantial variation in composition. In fact, the compositions overlap those of many other non-fibrous and fibrous minerals. Lee[4] has demonstrated that some specific amphiboles and related minerals can be discriminated by quantitative interpretation of SAED patterns obtained by tilting the fiber so that one of its principal crystallographic axes (zone axes) is parallel to the electron beam direction. However, demonstration that such patterns are compatible with the structure of a particular mineral does not exclude the possibility that they may also be compatible with the structures of other minerals. Only when the patterns have been shown to be incompatible with all other minerals in contention can it be stated that the fiber has been identified. Precise and well-established methods for unequivocal identification of amphibole asbestos in the TEM have not been reported in the literature.

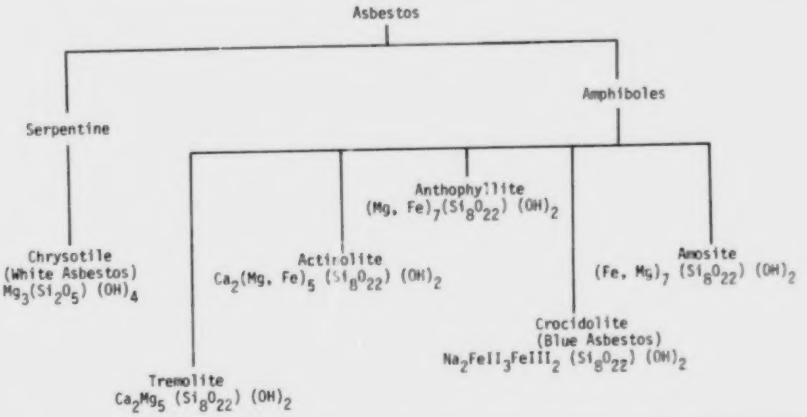
Asbestos
Serpentine
Amphiboles
Chrysotile
(White Asbestos)
$Mg_3(Si_2O_5)(OH)_4$
Tremolite
$Ca_2Mg_5(Si_8O_{22})(OH)_2$
Actinolite
$Ca_2(Mg, Fe)_5(Si_8O_{22})(OH)_2$
Anthophyllite
$(Mg, Fe)_7(Si_8O_{22})(OH)_2$
Crocidolite
(Blue Asbestos)
$Na_2FeII_3FeIII_2(Si_8O_{22})(OH)_2$
Amosite
$(Fe, Mg)_7(Si_8O_{22})(OH)_2$

Figure 1. Principal varieties of asbestos.

For the purposes of routine analyses of environmental samples, nearly all analysts have limited their identification procedure to observation of fiber morphology and simple visual inspection of an SAED pattern obtained from the fiber in whatever orientation it was found on the TEM specimen. On the basis of these observations, fibers were classified as chrysotile or amphibole, using known chrysotile and amphibole fibers as reference standards. Although this procedure is probably adequate in many circumstances for identification of chrysotile, it is in no way specific for the classification of fibers as amphibole.[5] Nevertheless, many analyses have been performed in which a layer-type SAED pattern with a 0.53 nm spacing has been taken as evidence for the presence of amphibole. However, the more cautious analysts using this approach have also used qualitative EDXA examination to supplement their observations, even if these were not specifically called for in the published analytical procedures. Even with this more cautious approach, the precise identity of fibers classified as amphibole by these routine methods is open to serious question, and more sophisticated techniques are required for unequivocal identification of fibers.

Methods for preparation of water samples for examination in the TEM have been the subject of considerable discussion. Of the methods that have been used, many have been discarded on account of particle losses during preparation. One study reported an inter-laboratory disagreement by a factor of 300.[6] The ideal TEM specimen for asbestos fiber counting has an absolutely uniform deposit of particulate over the whole grid, and the density of the particulate deposit is such that a significant degree of particle overlap does not occur. The supporting substrate for the particulate should also be featureless, and should not interfere with identification of the particles. A further requirement is that a specific area of the TEM specimen should be related in an accurate, quantitative manner to a known volume of the original water sample. Recently, the methods used for preparation of TEM specimens have fallen into several broad categories:

(a) Direct Transfer Methods

These methods are based on filtration of the water sample through a membrane filter, after which the filtered particulate is transferred to a carbon-coated TEM grid by solvent dissolution of the filter. In general it is found that particulate is lost or moved during the dissolution of the filter.[7]

(b) Replicated Filter Surface Methods

In the carbon-coated Nuclepore procedure,[2] a 0.1 μm pore size capillary pore polycarbonate membrane filter is used to filter the water sample. The active surface of the filter is carbon coated by vacuum evaporation, after which the filter is dissolved away in chloroform. The particulate is retained in position by the carbon film during the dissolution.[7] The conventional mixed-esters membrane filter has too much surface structure for this method to be directly applicable. However, after filtration of the water sample through such a filter, a method described by Ortiz and Isom[8] can be used to prepare a TEM specimen. The filter surface is exposed to acetone vapor, which causes the sponge structure to collapse into a continuous film. The assumption is made that the particulate is still at the surface after this operation. The procedure is then the same as for the carbon-coated Nuclepore preparation, except that acetone is used as the solvent.

(c) Drop Methods

In these methods the TEM specimen is prepared by evaporation of a drop of liquid on a carbon-coated support grid. There are several variations of this basic technique, but the common feature of all versions is the initial requirement for concentration of the fibers. Direct preparation from the water sample is not possible, since only 5 microliters of the liquid can be conveniently mounted in a single drop on a 3 mm diameter carbon-coated electron microscope support grid. If the concentration of asbestos in the original water is 10^6 fibers/liter, only 5 fibers would be transferred to the grid in this volume. Even concentration by a factor of 100, e.g. filtration of 200 mL followed by redispersal of the fibers in a 2 mL volume, results in the transfer of only 500 fibers unless multiple drops can be satisfactorily deposited on the grid. Moreover, much of the deposit is obscured by the grid bars which may occupy as much as 60% of the total grid area.

For the concentration step, ultra-centrifugation or filtration may be used. Ultra-centrifugation of liter volumes of water, and redispersal of the centrifugate in a smaller volume of clean water is a technique widely used in other fields, but no work has so far been reported in which the asbestos fiber losses have been compared with known low-loss preparations. Cross-contamination is undoubtedly a problem, but the technique does have the advantage that the use of filters is completely avoided along with their associated quality control deficiencies. The alternative approach is filtration, and in order to redisperse the particulate in a small volume of liquid, either the filter must be ashed or the particulate must be ultrasonically detached from it. Ashing is undoubtedly the superior approach, since ultrasonic detachment leaves a doubt as to how much material remains on the filter. Some results have been reported[9] which indicate that amphibole fiber losses during low temperature ashing are insignificant. No information is available for chrysotile fiber losses, apart from that of Chatfield et al.[7] The question then arises as to what proportion of the droplet volume is represented by the particulate deposit on one grid opening. If the droplet does not spread over the entire area of the grid before it evaporates, the actual area of particulate deposit is not known and it is also difficult to measure. Moreover, it is not likely that the density of particulate deposits in areas near the edge of the evaporated droplet is the same as in those areas close to the center.

As a first step in standardizing a satisfactory analytical method, a preliminary version of an interim procedure was published by the EPA in 1976.[10] It has since been shown that the carbon-coated Nuclepore technique included in this published procedure does not incur significant particle losses,[7] and that the uniformity of particle deposition is superior to that yielded by other methods tested. In contrast, this work[7] also indicated that direct transfer techniques involving reflux washing of unfixed particles on membrane filters were at best critically dependent on the skill of the operator, if not totally irreproducible. The non-reproducible behavior was observed for both the Jaffe Washer and the condensation washer. In light of these results, the membrane filter-condensation washer approach was deleted from the revised version of the Interim Procedure published in 1980.[2]

The carbon-coated Nuclepore procedure has been widely accepted as the most satisfactory current preparation technique, but no complete statistical studies have been performed using samples from real sources, as opposed to laboratory-prepared fiber dispersions. Even in this case, when chrysotile fiber dispersions in distilled water were analyzed by several laboratories, the results indicated that there was an unacceptable degree of variability compared with the results obtained when prepared filters were distributed.[11] It has also been found that there are some deficiencies in the Interim Procedure when analyzing real water samples, particularly in the case of organically contaminated waters. Although low temperature ashing is recommended in this procedure and by others,[12,13,14] even the initial filtration sometimes cannot be performed satisfactorily. In more marginal cases the absence of replicated filter detail indicates that there was a coating of slime on the filter surface. Any fibers embedded in this slime would not be transferred to the TEM samples, and consequently the results from such samples are of questionable accuracy. The author has previously reported application of a technique using ozone gas and ultraviolet light for oxidation of organic materials in liquid samples.[15,16] The treatment is given prior to the filtration, and in most cases interfering organic materials are oxidized to leave a clear liquid which is readily filtered. No other chemicals are used. Since the oxidizing agent is a gas, any amount of oxidizer can be applied to a sample without the addition of foreign ions or any increase in the total sample volume which would result in dilution of the original fiber concentration. Although the effects of the technique have not been fully and systematically investigated, it allows the use of the low temperature asher and high power ultrasonic treatment to be avoided. The technique does not remove large cellulose fibers; other similar refractory organics may also be incompletely oxidized by the treatment.

The research program directed towards improvement of the TEM analytical technique is described in this report. Sample collection and handling procedures were investigated, and various techniques for preparation of TEM specimens from water samples were compared. Particular attention was given to the problem of precise fiber identification and a standardized reporting format was developed. Computer programs were written to simplify the data reduction for both fiber identification and reporting. This work forms the background study supporting the issue of a new analytical technique for determination of asbestos in water samples, published separately.[17]

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The investigation of specimen preparation techniques for asbestos fiber counting by transmission electron microscopy showed that the carbon-coated Nuclepore method was superior to both the "drop" method and the collapsed membrane filter method. The "drop" method, in which a microliter volume of a concentrated dispersion is evaporated on a carbon-coated TEM grid, was shown to produce samples on which the fiber distribution was not sufficiently uniform to warrant their use in quantitative determinations. The collapsed membrane filter method was investigated in detail, and was shown to display strongly size-dependent fiber losses relative to the Nuclepore preparation. For chrysotile, the fiber losses increased with the pore size of the membrane filters used. For the 0.45 μm and 0.22 μm pore size filters, the losses were statistically significant at the 5% level; for the 0.1 μm pore size filters, fiber losses were not significant at the 5% level. For fibers shorter than 1.0 μm, the fiber losses using the 0.45 μm pore size filter were very high, and only 24% of these fibers were transferred to the TEM sample. The corresponding value for the 0.22 μm pore size filter was between about 60% and 70%. The results were consistent with the postulate that the shorter fibers penetrated the filter structure more deeply and were engulfed during the collapsing procedure. The results for crocidolite were more difficult to interpret. Although the total fiber losses using the 0.22 μm and 0.1 μm pore size filters were not significant at the 5% level, in some fiber size ranges significant losses were observed. On the basis of the results, the collapsed membrane method was found to be unsatisfactory for quantitative analyses. It is, however, recommended that if cellulose ester membrane filters must for some reason be used, plasma etching of collapsed membranes should be investigated as a means of increasing the transfer efficiency of short fibers to the TEM specimens.

Samples from water sources contaminated by chrysotile fibers were collected from Sherbrooke, Quebec, and it was shown that 10 replicate measurements from each of these samples were statistically compatible. The same conclusion was drawn for samples contaminated by amphibole fibers collected from the Duluth area of Minnesota. This indicated that for a series of sub-samples filtered at the same time, intra-laboratory measurements by a single operator using the same instrument were repeatable.

A method of oxidation of organic materials in water samples, based on the use of ozone gas and short wavelength (254 nm) ultraviolet light, was found to be successful. This oxidation technique was found to remove those organic components of drinking water samples which inhibit filtration, and was an effective and more convenient replacement for oxidation by two-step filtration and low temperature ashing. When the ozone-ultraviolet light technique was used, no changes in either the electron diffraction behavior or the chemical compositions of chrysotile and amphibole fibers were detected. A second oxidation technique, based on the use of oxygen at pressures up to about 13.8 MPa and temperatures of up to 300°C, was found to be effective for removal of organic materials, but some degradation of chrysotile fiber morphology was observed after treatment at the most extreme conditions. It was also found that containers made of polytetrafluoroethylene were required, since both glass and silica were attacked under the extreme conditions used. Because of the success of the ozone-UV technique, no further investigation of the more involved high-pressure method was conducted. However, it is recommended that for samples containing large concentrations of refractory organics, such as sewage or plant effluents, the technique warrants further consideration.

Studies of the stability of asbestos fiber dispersions yielded some surprising results. Initial experiments indicated that mechanical shaking of polyethylene bottles containing chrysotile fiber dispersions in double-distilled water reduced the suspended fiber concentrations to very low values. This effect did not occur if the bottles were exposed to continuous ultrasonic agitation for a similar period of time. The behavior was unaffected by either ionic or pH conditions. The effect was also observed for dispersions of crocidolite.

It was deduced that the presence of trace organic materials of bacterial origin in some way promoted the attachment of asbestos fibers to the inside surfaces of the containers. It was considered that this effect could seriously compromise the results of routine sample analyses. Container and storage studies were conducted which indicated that the effect was a consequence of an organic product of bacteria, rather than a mechanism involving direct interaction with the bacteria themselves, and that the organic material was probably a variety of polysaccharide. It was found that the effect of this phenomenon on routine sample analyses could be eliminated by ozone-UV treatment carried out inside the original sample container. This treatment was found to perform the double task of oxidation of interfering organic materials and release of fibers attached to the container.

The observation of the interaction of asbestos fibers with the trace organic materials had two other consequences: the development of stable reference fiber suspensions, and development of a separation technique which was at least partially specific for chrysotile. Reference fiber suspensions have been required for some time in order to facilitate analytical quality assurance programs, but their stability has always been in question. It was found that if the reference dispersions were prepared so as to exclude all bacteria and their organic products, they were then stable for long periods of time provided that absolute sterility was maintained. It is recommended that a standards agency maintain a supply of these reference dispersions, with appropriate certification of their contents, so that analytical quality assurance of future sampling programs can be established by incorporation of control samples.

Initial investigations of a separation technique based on fiber attachment to container walls were carried out as support for development of the rapid screening method. The separation technique was found to be effective as a selective method for concentration of chrysotile asbestos fibers from water samples; its selectivity for separation of amphibole fibers was not tested. It is recommended that this separation method should be developed further, and that the mechanism which gives rise to the attachment phenomenon should be investigated. The observation of this strong interaction between asbestos fibers and organic materials of biological origin may have significance in other fields unrelated to analytical method development.

The fiber identification protocol based on zone axis SAED and quantitative EDXA is capable of more specificity than had previously been provided by TEM analysis. The identification procedure permits determination of approximate chemical composition, which is adequate for the general classification of amphibole fibers, but is not sufficiently precise for the incorporation of adjectival modifiers in the mineral description. It is recommended that the identification procedure be reviewed on a regular basis, and that more precise X-ray analytical procedures be developed and applied as they become available.

The improved reference analytical method for determination of asbestos fibers in water represents the best available technology. A number of new features were incorporated. These include the introduction of ozone-UV oxidation for all samples, a fiber classification system, a minimum fiber length for reporting, a standardized reporting format, quantitative interpretation of fiber identification data, and a fibrosity index which appears to permit discrimination of fibrous and non-fibrous species. The changes and additions introduced into the basic analytical method should eliminate the problems of poor interlaboratory reproducibility which have been observed in the past.

Conclusions and recommendations relating to the development of a rapid screening technique for measurement of asbestos concentration are included in the separate report on this aspect of the overall program.

SECTION 3

INVESTIGATION OF TEM SAMPLE PREPARATION TECHNIQUES

Although application of the EPA Interim Method[2] has apparently been successful, both before and since its publication a number of other preparation techniques have been used by some laboratories. These techniques include several variations of the "drop" method and the collapsed membrane filter method.

A serious problem associated with the carbon-coated Nuclepore method (recommended in the Interim Method) is the insolubility of some components of the filter in organic solvents. This undesirable feature of the polycarbonate filter is associated with the polymer crystallinity, and is actually both necessary and intentionally present so that the membrane has adequate strength. Unfortunately this property appears difficult to control during manufacture and is variable between individual batches of filters. The replication of the filter surface and the pores has also been considered to be a minor inconvenience, since the surface detail creates a distraction for the microscope operator during TEM examination. However, when samples with very little deposit on the filter surface are examined, the replicated filter detail is useful in that it provides sample position reference for the operator. The background detail in these circumstances is therefore an advantage. The problems associated with limited filter solubility can be avoided by using either the "drop" technique or the collapsed membrane filter technique. These techniques also yield TEM samples with reduced surface detail.

The direct "drop" method consists of concentration of the fibers by filtration or centrifugation, followed by deposition of a few microliters of the concentrated dispersion on a carbon or collodion-coated TEM grid. There are a number of variations of this method,[12,18] but the initial requirement is one of sample concentration. Direct preparation from the water sample is usually not possible, since only 5 microliters of the liquid can be conveniently mounted in a single drop on a 3 mm diameter carbon-coated electron microscope support grid. The level of asbestos in most waters is too low to provide a sufficient number of fibers in the 5 μL drop. If filtration is used to concentrate the sample, the filtered particulate must be removed from the filter and redispersed in a small volume of liquid before the grid can be prepared. The only routes currently available for this are ashing of the filter, or ultrasonic detachment of the particulate. Alternatively, centrifugation has sometimes been used to concentrate the sample. Although ultra-centrifugation of liter volumes of water followed by redispersal of the centrifugate in a smaller volume of clean water is a technique widely used in other fields, no work has so far been reported in which the asbestos fiber losses have been compared with known low-loss preparations. However, this technique does avoid completely the use of filters and any associated quality control deficiencies.

When the concentrated suspension has been prepared, a few microliters are then transferred to a coated grid. The question of how uniform the deposit will be after evaporation has been discussed extensively. Mudroch and Kramer[18] used a carbonized collodion-coated grid and took no action to facilitate spreading of the droplet, whereas Cunningham and Pontefract treated the carbon-coated grid for a short period in a low temperature plasma asher to render its surface hydrophilic.[19] Moreover, the proportion of the drop represented by the deposit on one grid opening must be accurately determined before the concentration of fibers in the original sample can be calculated.

Another technique which has been used by some laboratories is the "collapsed membrane" method.[8] Essentially, the liquid is filtered through a conventional mixed-esters sponge-type membrane filter, after which the filter structure is collapsed into a continuous film by exposure to solvent vapor. For TEM sample preparation the filter surface is then carbon coated, after which the filter is dissolved by a Jaffe Washer procedure using acetone as the solvent, leaving an extraction replica which contains the particles which were originally on the surface of the filter. The technique requires that all of the fibers remain on the surface of the filter after the collapsing process, so that they are available for entrapment in the evaporated carbon film; any particles which become covered during collapse of the structure will not be transferred to the TEM specimen. Particle losses using this technique are likely to be most serious for the larger pore-size filters, on which fibers can penetrate more deeply into the filter structure during sample filtration.

3.1 DROP METHOD

A short study was made of the characteristics of TEM grids prepared by the "drop" technique. The uniformity of the particulate deposit on the grid openings was investigated using several of the technique variations which have been published.[18,19,20] The problem of uniformity of deposit was considered by Cunningham and Pontefract,[19] who observed that the carbon film could be rendered hydrophilic by placing the grids in a plasma asher for a few minutes. The Ontario Ministry of the Environment[20] attempted to solve this problem by adding detergent to the concentrated fiber dispersion before mounting the drop on the grid. The merits of inverting the grid during the evaporation of the sample drop have also been considered.

Carbon-coated copper grids were prepared by the film cast technique in which a drop of 1% collodion in n-pentyl acetate is placed on water in a 10 cm diameter crystallizing dish. After the solvent has evaporated, copper grids are dropped onto the floating collodion film. A Whatman filter paper is then placed on the floating film, and immediately removed and inverted. The collodion film adheres to the filter paper, trapping the grids. After the filter paper is dry, the collodion-coated grids can be removed. The grids are then carbon-coated by vacuum evaporation, and the collodion layer is dissolved away by reflux washing with acetone in a Jaffe Washer.[21]

A standard dispersion of UICC chrysotile was used as the test dispersion for mounting on the grids. Grids were prepared from the test dispersion as follows:

(a) untreated grids, evaporation both upright and inverted;

(b) plasma-treated grids, evaporation both upright and inverted;

(c) untreated grids, detergent added to dispersion, evaporation both upright and inverted.

Grid openings were selected randomly and the number of fibers on each was recorded. A chi-square test was used to test the uniformity of the deposit on 10 grid openings of each grid.

None of the grids displayed a uniform deposit of fibers at even the 0.1% significance level. Because of this observation, further work on the "drop" methods of TEM grid preparation was not carried out. Although these methods are convenient for mounting small quantities of particulate material where quantitation is not required, they are not suitable for quantitative fiber concentration measurements.

3.2 COLLAPSED MEMBRANE FILTER METHOD

A significant feature of the carbon-coated Nuclepore technique is that during the filter dissolution step, all of the particles on the filter surface are held in position by the evaporated carbon film, resulting in a minimal loss of particulate during the preparation. The technique described by Ortiz and Isom[c] apparently permits the same advantage to be obtained when a conventional mixed-esters membrane filter is used for the sample filtration. In the technique of Ortiz and Isom, the sponge structure of the filter is collapsed into a thin plastic film by exposing the filter to acetone vapor. The steps in the procedure are shown in Figure 2. The exposure to acetone vapor must be monitored very carefully,

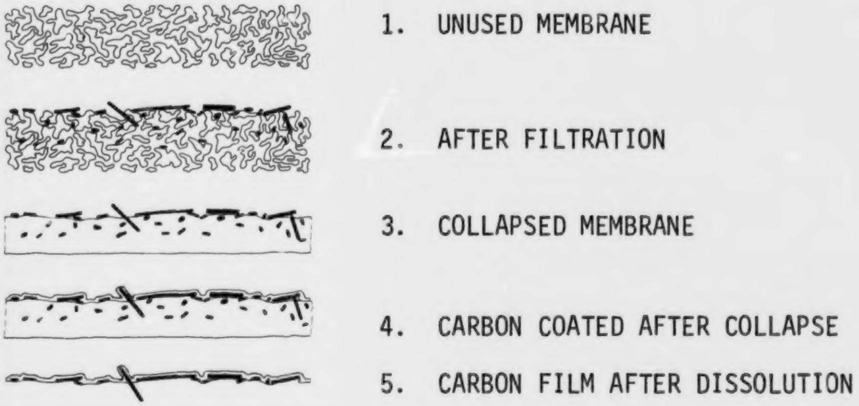
1. UNUSED MEMBRANE
2. AFTER FILTRATION
3. COLLAPSED MEMBRANE
4. CARBON COATED AFTER COLLAPSE
5. CARBON FILM AFTER DISSOLUTION

Figure 2. Steps in the collapsed membrane procedure of filter preparation.

since under-exposure will fail to collapse the surface structure to a continuous film, and over-exposure may cause the fibers to become engulfed in the plastic. Although chromium was incorporated with carbon as the evaporated coating in the published method, this would be unsuitable for application to asbestos measurement because it would yield a strong diffraction pattern of its own during examination of fibers by SAED. The coating material is not considered to be critical, (Ortiz, L.W., personal communication) and carbon was selected.

In an initial study, a series of suitably loaded filters was prepared by filtration of identical volumes of a dispersion of chrysotile. The fiber size distribution was selected to be similar to that normally encountered in water samples. The filters used were:

(a) 0.1 μm pore size Nuclepore capillary pore polycarbonate;

(b) 0.1 μm pore size Millipore mixed cellulose esters;

(c) 0.22 μm pore size Millipore mixed cellulose esters; and

(d) 0.45 μm pore size Millipore mixed cellulose esters.

The filters were prepared by repeating the sequence (a) to (d) until 5 filters of each type were prepared; in this way any change in the fiber concentration of the dispersion during the preparation would affect all of the filter types equally. When using the collapsed membrane technique, a 50% success rate in preparing specimen grids suitable for TEM fiber counts was achieved. Some filters disintegrated immediately upon being placed in the Jaffe Washer. In two cases a film of plastic remained which was sufficient to interfere with fiber identification even after the grids had been on the Jaffe Washer for 17 hours. There was always a significant degree of breakage of the carbon film during the filter dissolution step; and in some cases only two or three grid openings remained intact on the grid. So far as the removal of filter structure detail is concerned, it is considered that the best results were obtained when the sample was exposed to acetone vapor during the collapsing step until the filter just cleared.

The results obtained during the initial study are shown in Table 1. The detailed fiber counting data are shown in Table A-1 of Appendix A. In Table 1, comparison of the values obtained from the membrane filters with those from the carbon-coated Nuclepore filters shows a progressively increasing degree of fiber loss as the membrane filter pore size increases. At the 5% significance level, statistically valid numerical fiber losses of 20.4% and 56.5% are shown for the 0.22 μm and 0.45 μm pore size Millipore filter preparations respectively.

A more detailed study was made on the performance of the 0.1 μm and 0.22 μm pore size Millipore cellulose esters membrane filters. In view of the initial results, no further work was performed on the 0.45 μm pore size membrane filters. Using a standard dispersion of refined Union Carbide Calidria chrysotile, filters were prepared as before, alternating

TABLE 1. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE: INITIAL MEASUREMENTS

Preparation	Number of Filters Analyzed	Fiber Concentration (10^6 Fibers/Liter)		Fiber Loss in Preparation (%)*	Is the Fiber Loss Detectable at 5% Significance?
		Mean	95% Confidence Interval		
Carbon-Coated Nuclepore 0.1 µm Pore Size Polycarbonate	3	26.9	20.4 - 33.4	-	-
Collapsed Millipore 0.1 µm Pore Size Cellulose Esters	3	26.0	9.9 - 42.1	3.3	No
Collapsed Millipore 0.22 µm Pore Size Cellulose Esters	5	21.4	15.7 - 27.1	20.4	Yes
Collapsed Millipore 0.45 µm Pore Size Cellulose Esters	3	11.7	6.1 - 17.3	56.5	Yes

*Compared to the Carbon-Coated Nuclepore Preparation the types of filter throughout the series of filtrations. Ten filters each of the 0.1 µm pore size Nuclepore, and Millipore filters of 0.1 µm and 0.22 µm pore size, were prepared with identical fiber loadings. TEM specimen grids were prepared from the Nuclepore filters by the conventional carbon coating procedure, and from the Millipore filters by the collapsing technique. The results are shown in Table 2, with the detailed fiber counting data in Tables A-2 to A-4 of Appendix A. It can be seen in Table 2 that the 0.1 µm Millipore filter preparations showed no detectable fiber loss at 5% significance, but that the 0.22 µm Millipore preparations showed a mean loss of 23.4% relative to the Nuclepore preparations.

The fiber losses of the collapsed Millipore preparations might be expected to be fiber size-dependent, since they are probably a consequence of embedding of the deposit during the collapsing process. If the carbon-coated Nuclepore technique is assumed to incur negligible fiber losses, the fiber length distributions observed using the two techniques allow the fiber transfer efficiency of the collapsed membrane technique to be calculated as a function of fiber length. These transfer efficiency values are independent of the fiber length distribution of the dispersion used for the comparison. The transfer efficiency histograms for chrysotile, using 0.45 µm, 0.22 µm and 0.1 µm pore size membrane filters, are shown in Figure 3. It can be seen that for the 0.1 µm pore size membrane filter, the transfer efficiency remained at about 100% for the entire fiber length range investigated (0.2 µm to 10 µm). In contrast, the results using the 0.45 µm pore size membrane filters were unsatisfactory; for fiber lengths shorter than 1 µm, only 24% of the fibers were transferred to the TEM grids. The results using the 0.22 µm pore size membrane filters roughly paralleled those for the 0.45 µm pore size filters, but they showed somewhat higher transfer efficiencies. It is clear that a significant proportion of the fibers failed to transfer to the TEM grids when the 0.22 µm and the 0.45 µm pore size filters were used, and that this effect was strongly fiber size-dependent. Accordingly, the collapsed membrane filter technique using the larger pore size filters introduces a serious distortion into the observed fiber length distribution.

To test the collapsed membrane preparation technique using amphibole fibers, a similar series of filter samples was prepared using a dispersion of UICC crocidolite. The results obtained are shown in Table 3, with the detailed fiber counting data in Tables A-5 to A-7 of Appendix A. In Table 3 it can be seen that statistically significant total fiber losses were not demonstrated for either the 0.1 µm or the 0.22 µm pore size Millipore filter preparations. Figure 4 shows the fiber transfer efficiency histograms, which indicate that the behavior of crocidolite was different from that of chrysotile. In particular, for both the 0.1 µm and the 0.22 µm pore size filters, the longer fibers were transferred less efficiently to the TEM specimen grids. The histograms show that substantial distortions were introduced into the observed fiber length distributions.

TABLE 2. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE

Preparation	Number of Filters Analyzed	Fiber Concentration (10^6 Fibers/Liter)		Fiber Loss in Preparation (%)*	Is the Fiber Loss Detectable at 5% Significance?
		Mean	95% Confidence Interval		
Carbon-Coated Nuclepore 0.1 μm Pore Size Polycarbonate	10	264.8	236 - 293.6	-	-
Collapsed Millipore 0.1 μm Pore Size Cellulose Esters	10	285.0	246.4 - 323.6	0	No
Collapsed Millipore 0.22 μm Pore Size Cellulose Esters	10	202.8	180.5 - 225.1	23.4	Yes

*Compared to the Carbon-Coated Nuclepore Preparation

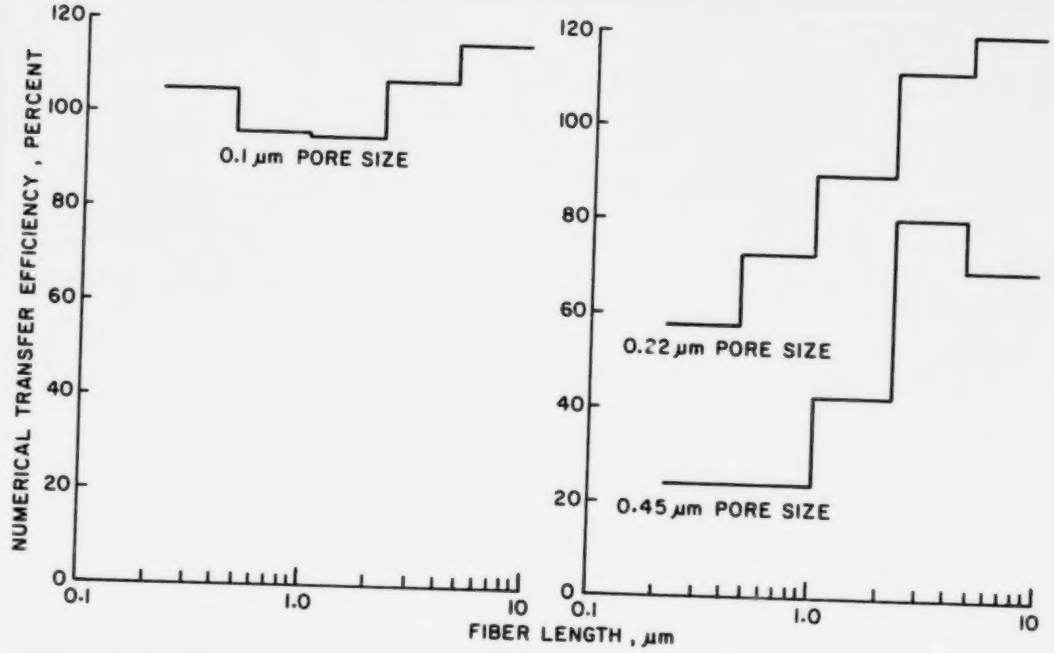

Figure 3. Collapsed membrane filter method: Chrysotile transfer efficiencies.

TABLE 3. COLLAPSED MEMBRANE STUDY FOR CROCIDOLITE

Preparation	Number of Filters Analyzed	Fiber Concentration (10^6 Fibers/Liter)		Fiber Loss in Preparation (%)*	Is the Fiber Loss Detectable at 5% Significance?
		Mean	95% Confidence Interval		
Carbon-Coated Nuclepore 0.1 µm Pore Size Polycarbonate	10	224	158 - 290	-	-
Collapsed Millipore 0.1 µm Pore Size Cellulose Esters	10	229	200 - 258	0	No
Collapsed Millipore 0.22 µm Pore Size Cellulose Esters	10	204	183 - 225	8.9	No

*Compared to the Carbon-Coated Nuclepore Preparation

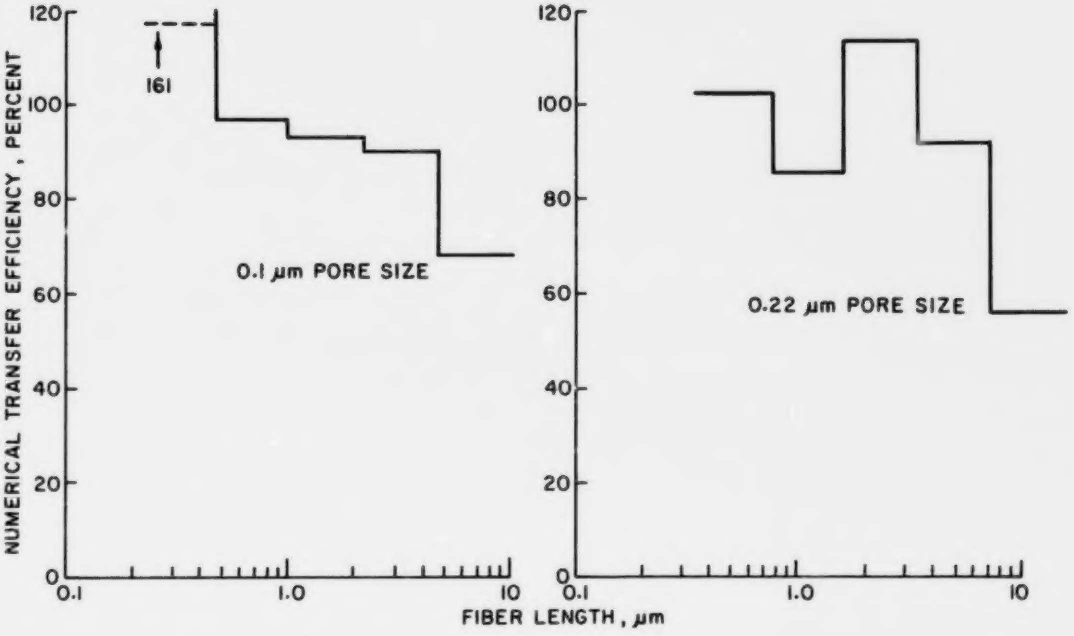

Figure 4. Collapsed membrane filter method: UICC crocidolite transfer efficiencies.

3.3 SUMMARY OF ALTERNATIVE SAMPLE PREPARATION METHODS

The purpose of the investigation was to determine if any other TEM preparation technique would give results superior to those of the carbon-coated Nuclepore method, or if a simpler technique of equivalent performance could be defined. The "drop" methods were found to be quite unsatisfactory, since uniform deposits could not be obtained reliably. Moreover, because the area over which the drop spread before it evaporated is not easily measured, it is difficult to determine an accurate relationship between the fiber count on a small number of grid openings and the fiber concentration of the suspension being investigated. Accordingly, the technique was not investigated further.

The collapsed membrane technique appeared initially to be promising, but it was found that only a small proportion of the grid openings had carbon film which was intact, and that the success rate for satisfactory grid preparation was only about 50%. The technique appeared to be equivalent in performance to the carbon-coated Nuclepore method for chrysotile deposited on 0.1 μm pore size filters. For larger pore sizes, the method had significant size-dependent fiber losses. For amphibole fibers, total numerical fiber losses were not demonstrated for either 0.1 μm or 0.22 μm pore size Millipore filters, but it appears that there was a trend towards lower transfer efficiencies for the longer fibers on filters of both pore sizes.

SECTION 4

REPRODUCIBILITY OF ENVIRONMENTAL WATER SAMPLE ANALYSES

4.1 GENERAL

Although satisfactory reproducibility has been demonstrated for analyses of a large number of Nuclepore filters which were prepared at one time from a single fiber dispersion,[7,22] this has not been the case when a water sample was analyzed at different times after it was collected. Inter-laboratory analyses conducted by distribution of water samples, rather than filters, have largely been unsuccessful.[11] These studies have displayed significant variability for both well-controlled fiber dispersions prepared in the laboratory and naturally-occurring fiber dispersions. Moreover, collection of separate samples from the same source has sometimes yielded conflicting results. A study was made to determine the intra-laboratory analytical reproducibility of several naturally-occurring fiber dispersions. The study was designed so that any major effect of sample volume on the analytical reproducibility could also be determined.

Preliminary work indicated that fiber dispersions vary greatly in their ability to yield satisfactory TEM samples. Natural sources can often produce a very non-uniform, highly agglomerated deposit on the TEM specimen. Inorganic particles tend to agglomerate with the gelatinous organic matter sometimes found in natural waters. This effect is illustrated in Figure 5, which shows the deposit formed on a Nuclepore filter by the filtration of 20 mL of water from an Ontario lake which is known to have high levels of chrysotile contamination. The sample was shaken vigorously for about 30 minutes before the aliquot was taken. As can be seen in the micrograph, the suspended matter has not been uniformly dispersed. A TEM sample prepared in this way would not allow identification of most of the chrysotile which is present. Figure 6 shows the deposit from 20 mL of the same water sample, but the sample had been exposed to ultrasonic treatment at a power of 2.2 W/L for a period of 30 minutes before filtration. The deposit would still not permit identification of most fibers mixed with it. This problem has been found to occur frequently in raw lake and river waters, particularly after storage of the samples. In these cases, it is very difficult to produce a uniform, homogeneous suspension which will permit the preparation of useful specimens directly by the carbon-coated Nuclepore technique. Prior to these studies, the factors which control the stability of an asbestos fiber dispersion were not clearly understood. Similar phenomena had been observed even for simple dispersions of chrysotile in distilled water, although on some occasions such dispersions were found to be stable for long periods of time.

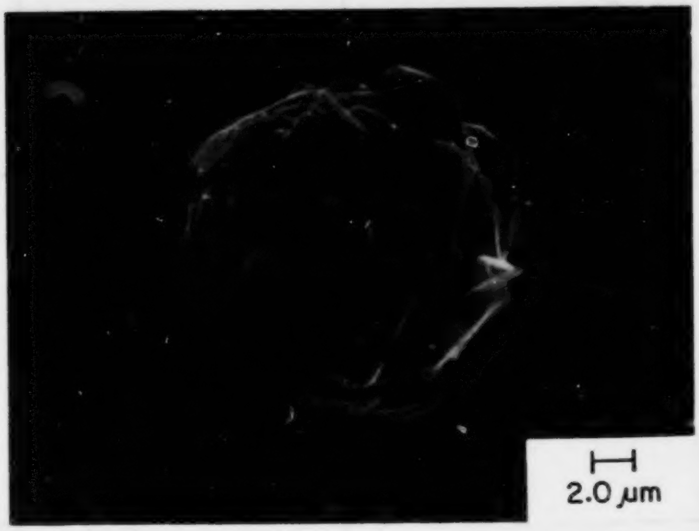

Figure 5. SEM micrograph of the deposit on a Nuclepore filter produced by filtration of 20 mL of lake water after the sample was shaken.

Figure 6. SEM micrograph of the deposit on a Nuclepore filter produced by filtration of 20 mL of lake water after ultrasonic treatment at 2.2 W/L for 30 minutes.

The volume of sample required in order to ensure that the sample is representative of the source concentration is probably related to the character of the source. It can readily be demonstrated that samples collected sequentially from a faucet may yield statistically incompatible values for the fiber concentration, whereas the values obtained from replicate samples taken from the same bottle may be compatible. Table 4 shows the chrysotile concentrations found in a study of the Sherbrooke, Quebec water supply,[23] and illustrates the limitation of "grab" samples. Although analyses of replicates from the same bottle were statistically compatible, the actual range of values obtained at the different points in the system indicates either a temporal variation or source inhomogeneity on the one liter scale.

TABLE 4. CHRYSOTILE FIBER CONCENTRATIONS IN MUNICIPAL DRINKING WATER, SHERBROOKE, QUEBEC

Water Type	Fiber Concentration (10^6 Fibers/Liter)	
	Mean	95% Confidence Interval
Raw	72.5	59.1 - 85.9
Treated*	26.1	16.3 - 35.9
Distribution 1	222	177 - 267
Distribution 2	80.3	30 - 129

*Municipal water treatment, at the time of sampling, consisted only of the addition of chlorine gas and ozone to the raw water.

A study was undertaken to evaluate the reproducibility of the EPA Interim Method[2] when applied to environmental samples, and to investigate any observed container or sample volume effects. The analyses were made of samples collected from eight locations, four of which were associated with an amphibole fiber source and four with a chrysotile source.

4.2 SAMPLE COLLECTION

A sampling scheme was devised for the collection of environmental samples, both from tap sources and from large bodies of water. These samples were used in the investigation of:

(a) the reproducibility of analyses of replicates from one collected sample;

(b) the reproducibility of duplicate samples from a single source; and

(c) the determination of a suitable sample volume.

The sampling scheme for collection of tap water samples was to fill one 5 gallon polyethylene bottle, followed by alternate 1 liter and 250 mL bottles until thirty of each had been collected, and completing the series with another 5 gallon bottle. The sampling of a large body of water was subdivided into two sets. Two sets of samples were then taken from the body of water which serves as the raw water supply for the location in which the tap water samples were obtained. In the first set a total of 30 samples were collected, alternating between 1 liter and 250 mL sample bottles, followed by collection of a single 5 gallon sample. The second set of samples was collected in a similar manner at a sampling point in a water treatment plant supplied from the raw water source.

For water with high levels of amphiboles, tap water samples were taken in Beaver Bay, Minnesota (in the Duluth area). Here the water is taken from Lake Superior and pumped through a coarse sand filter directly into the distribution system. The raw water samples were taken at the shore of Lake Superior off a rocky promontory at the Buchanan Town Marker, about 11 miles northeast of Duluth. The final series of samples was taken in the Duluth Pumping Station. The raw water intake is stated to be about ¼ mile from the shoreline and 75 ft. below the surface.

Similar sets of samples were also collected in the Sherbrooke area of Quebec province where the river water and the municipal water were known to contain chrysotile fibers at concentrations adequate for this study. The tap water samples were collected in Sherbrooke. At the time of sampling, the municipal water was taken from the Magog River and treated with chlorine gas and ozone. There was no filtration of the water before distribution. The series of raw water samples was taken at the shore of the Magog River, approximately 1½ miles upstream of the treatment plant intake. Samples were also taken from the raw water intake at the Sherbrooke Water Treatment Plant.

4.3 ANALYTICAL REPRODUCIBILITY

Ten replicate filters were prepared from each of the eight 5 gallon bottles. These filters were analyzed to determine the analytical reproducibility of replicate samples prepared from natural waters known to contain either amphibole or chrysotile fibers. In the case of the Duluth area samples, it was found that the number of amphibole fibers found on 10 grid openings of the TEM specimens was inadequate for satisfactory analysis of the data. The level of total solids in the water did not permit filtration of larger volumes. Accordingly, the fiber counts were continued until 20 grid openings had been examined. For the Sherbrooke area samples, the relative levels of chrysotile and other materials were such that fiber counts on 10 grid openings yielded sufficient numbers of chrysotile fibers for analysis of the data.

The detailed analytical data are shown in Tables B-1 to B-8 of Appendix B. Statistical analyses have been made of the results, using the grid opening fiber counts. These analyses are also shown in Appendix B in Tables B-9 to B-16. For each series of replicate analyses, the mean value of the ten measurements has been calculated, along with an estimate of variance for the mean. Using the two-sided "t"-test, each individual sample analysis result has been compared with the mean of ten measurements, to determine if it is a replicate value of the mean. In the majority of cases, the results show that, at 5% significance, the individual values are indistinguishable from the mean. A summary of the statistical results is shown in Table 5. The study indicates that from a single 5 gallon sample, with few exceptions it is possible to obtain replicate results from 10 mL sub-samples, both for the amphibole and the chrysotile dispersions.

As has been found on previous occasions, subsequent analyses of the separately-collected 1 liter and 250 mL samples did not replicate the values obtained from the 5 gallon samples. The values obtained for the first samples analyzed were all significantly lower than those which had been obtained from the larger 5 gallon samples. No further analyses were performed on these samples, since it was considered more beneficial to investigate the reasons for the instability of such suspensions.

TABLE 5. REPLICATE ANALYSES OF ENVIRONMENTAL WATER SAMPLES: SUMMARY OF RESULTS

Source	Fiber Type	Number of Analyses Statistically Compatible with Mean*	Number of Samples Analyzed
Beaver Bay Tap Water, Initial Sample	Amphibole	9	10
Beaver Bay Tap Water, Final Sample	Amphibole	10	10
Lake Superior Water	Amphibole	10	10
Duluth Raw Water	Amphibole	9	10
Sherbrooke Tap Water, Initial Sample	Chrysotile	9	10
Sherbrooke Tap Water, Final Sample	Chrysotile	7	10
Magog River, Sherbrooke	Chrysotile	9	10
Sherbrooke Raw Water	Chrysotile	10	10

*Two-sided "t"-test at 5% significance

SECTION 5

OXIDATION OF ORGANIC MATERIALS

Organic materials, often present in water samples as slimes or algae, interfere with TEM specimen preparation by the carbon-coated Nuclepore procedure. A coating of slime on the filter has two effects: it retards or may even prevent filtration of the sample; and since only the top surface of the coating is replicated, any particles embedded in it are not transferred to the final replica which is examined in the TEM. Furthermore, these organic materials have been found to scavenge fibers from suspension. A common method for removal of the organic materials is the low temperature plasma ashing technique, as described in the EPA Interim Method.[2] However, in many cases the filter becomes blocked before a useful volume can be filtered, and then it is not possible to analyze the sample satisfactorily.

A new technique has been developed for removal of organic materials by the use of 1% ozone gas and exposure to short wave ultraviolet light.[15,16] Although primarily developed for, and applied to, the analysis of beverages such as wine and beer, the technique has been successfully applied to a number of industrial effluent samples and urine. The interfering organic materials in most water samples can be oxidized by treatment for a period of about 4 hours. However, the technique does not attack bulk cellulose rapidly, thus other methods must be used if cellulose is considered an interference. The advantage of the ozone-UV technique is that no acids or chemicals are added to the sample, and the equipment is both simple and inexpensive.

A different and more powerful oxidation technique was also investigated for application to those samples where large amounts of cellulose or other similar refractory organics are present. In this technique the water sample is exposed to oxygen gas at high pressure and at an elevated temperature. In neutral or alkaline conditions, complete oxidation takes place at about 13.8 MPa and 300°C, without the requirement for addition of other reagents. Suitable high pressure research reactors are available commercially. It is estimated that 2 samples per day could be processed in one reactor.

The advantages of the oxidation methods proposed are that no chemicals are added which might carry contamination, and that the procedures are very simple to perform. Using the ozone-UV technique, it has become relatively simple to analyze many liquid samples which, because of slow filtration, could not previously be prepared satisfactorily.

5.1 THE OZONE-UV OXIDATION METHOD

The required equipment is shown schematically in Figure 7; the system in

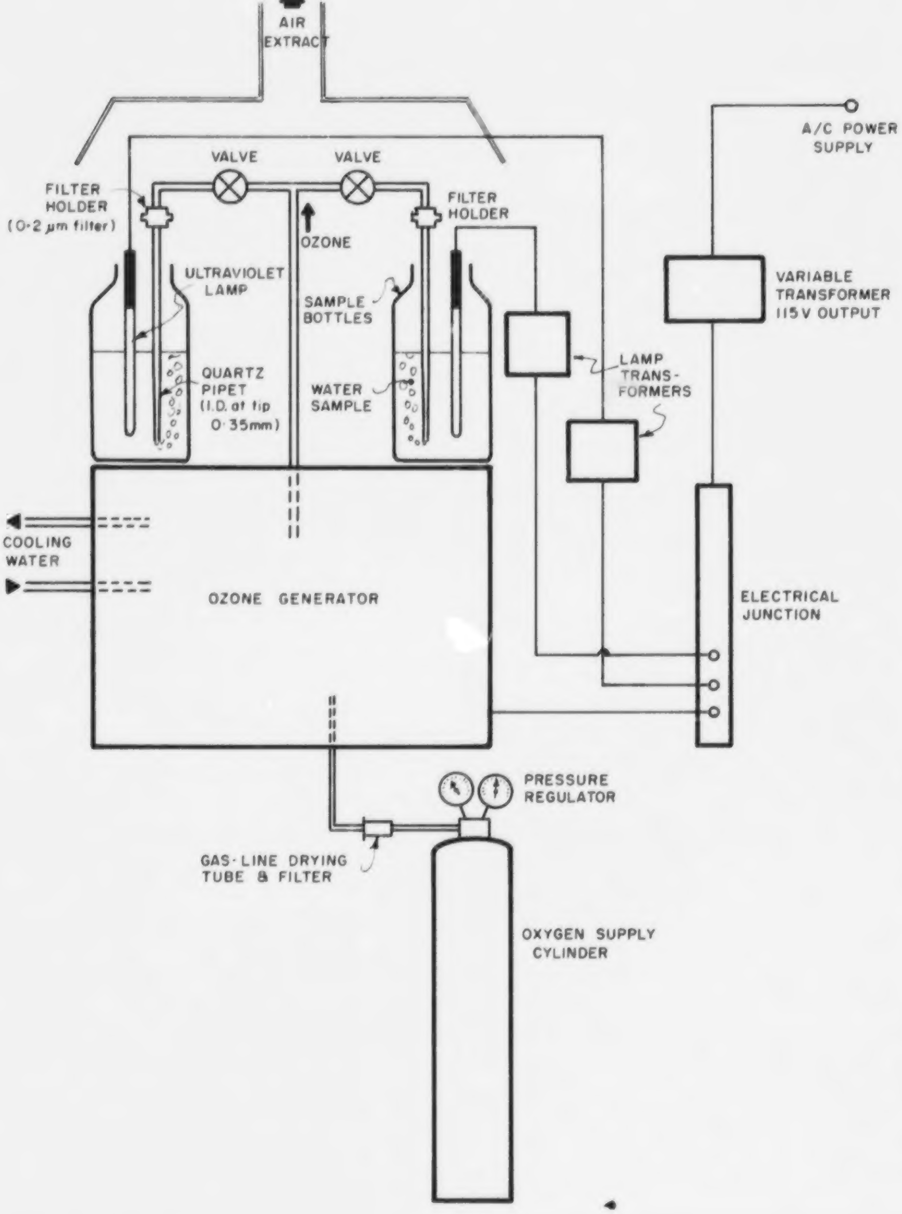
AIR EXTRACT
VALVE
VALVE
FILTER HOLDER (0·2 μm filter)
FILTER HOLDER
OZONE
ULTRAVIOLET LAMP
SAMPLE BOTTLES
QUARTZ PIPET (I.D. at tip 0·35mm)
WATER SAMPLE
A/C POWER SUPPLY
VARIABLE TRANSFORMER 115V OUTPUT
LAMP TRANS-FORMERS
COOLING WATER
OZONE GENERATOR
ELECTRICAL JUNCTION
PRESSURE REGULATOR
GAS-LINE DRYING TUBE & FILTER
OXYGEN SUPPLY CYLINDER

Figure 7. Equipment used for the ozone-UV oxidation technique.

use for simultaneous oxidation of two samples is shown in Figure 8. An air extract or fume hood is required to remove surplus ozone. Before being ozone-UV treated, the polyethylene or glass bottle containing the water sample is placed in an ultrasonic bath for a period of 15 minutes. The level of the liquid in the sample bottle is marked on the outside, using a felt marker. The quartz pipets (formed by drawing quartz tubing to the required length and tip diameter), are thoroughly washed before each use, and installed on the ozone supply, as indicated, so that the tip is close to the bottom of the sample bottle. The UV lamp is also thoroughly washed and then immersed in the sample and switched on.

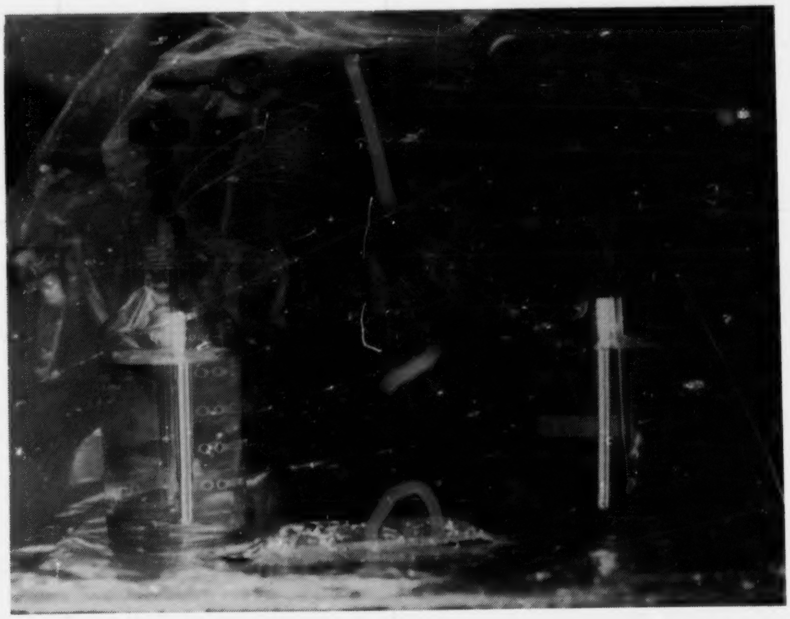

Figure 8. Ozone-UV oxidation of water samples in glass bottles. The ozone supply line has been split into two lines to permit simultaneous oxidation of two samples. A valve and a filter holder are incorporated in each of the supply lines to the samples.

At an ozone concentration of about 4% in oxygen, each sample is treated with about 1 liter/minute of gas for approximately 3 hours. At other ozone concentrations, the oxidation time should be adjusted so that each sample receives about 10 grams of ozone. The gas flow rate should be sufficient to produce a mixing action in the liquid but should not splash any of the sample out of the container. It is not easy to indicate when oxidation has been completed, but this treatment, as described, has been found to be adequate for all water samples so far analyzed. When oxidation is complete, the UV lamp and quartz pipet are removed after which the bottle is re-capped and placed in an ultrasonic bath for a period of fifteen minutes. This allows particulate released from the oxidized organic materials and the container surfaces to be uniformly dispersed throughout the sample. During the oxidation procedure, evaporation may result in a reduction of the sample volume. This loss of volume should be noted and, if significant, can be accounted for by either addition of distilled water or calculation. The sample should be filtered immediately after it is removed from the ultrasonic bath.

An initial evaluation of the ozone-UV oxidation technique was made using a dispersion of refined Union Carbide Calidria chrysotile. The organic material chosen for the test was a starch solution. After ozone-UV oxidation of a 0.1% starch solution, it was possible to filter 100 mL of the liquid. However, it was found that even a 0.001% solution of starch in double-distilled water could not be filtered through a 0.1 μm pore size Nuclepore filter. A volume of 4 mL of the Union Carbide chrysotile dispersion, which had a concentration of about 200 x 10^6 fibers/liter, was added to 96 mL of 0.1% starch solution. Four samples were prepared in this manner, and the standard ozone-UV technique was applied, followed by treatment in the ultrasonic bath for 15 minutes. The results obtained are shown in Table 6. A statistical analysis shows that at 5% significance, the ozone-UV treated sample results do not differ from those of the controls.

Further qualification of the ozone-UV technique was demonstrated using both artifically-prepared and naturally-occurring chrysotile dispersions. These results are discussed in the overall context of Section 6, which deals with sample container effects.

5.2 HIGH PRESSURE OXIDATION

The feasibility of oxidation of organic materials by high pressure oxygen was tested using a stirred reactor. For this purpose, several refractory organic dispersions in water were used. These were selected to be representative of materials which may be present in water supplies, rivers or lakes. The oxidations were carried out at a pressure of 4.8 MPa and a temperature of 250°C. The total pressure of 4.8 MPa comprised an oxygen partial pressure of 1.0 MPa and 3.8 MPa of steam. The reactor was initially charged with oxygen to a pressure of 0.7 MPa, and during the reaction time of 90 minutes the sample was stirred at 800 rpm. The experiments described below demonstrated that the procedure was very successful.

(a) Proteins

A slurry of ground beef, including the fats, was oxidized by the high pressure oxygen procedure, leaving a clear, yellow filterable liquid. Ozone-UV oxidation was unsuccessful for oxidation of this sample.

(b) Cellulose

A dispersion of cellulose was prepared by ultrasonic dispersal of

TABLE 6. RESULTS OF ANALYSES OF CHRYSOTILE DISPERSIONS IN 0.1% STARCH SOLUTION

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Squares	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Nanograms/Liter)	(10^6 Fib/Liter)			x^2	Significance of Uniformity (%)
CONTROL SAMPLES								
Control 1	165	87 - 244	2400	1.62	102	9	35.1	<0.1
Control 2	148	101 - 194	1720	1.51	98	9	14.4	5
Control 3	251	182 - 320	3150	2.28	110	6	6.3	25
OZONE-UV SAMPLES								
Ozone-UV 1	199	157 - 241	1610	1.84	108	4	1.4	50
Ozone-UV 2	140	110 - 170	1250	1.44	97	5	2.3	50
Ozone-UV 3	122	96.8 - 146	1640	1.11	110	7	4.6	50
Ozone-UV 4	117	81.8 - 152	893	1.22	96	6	6.7	10

two sheets of tissue. After the high pressure oxygen procedure a clear, colorless liquid remained which was filterable. The ozone-UV technique was not effective for cellulose oxidation.

(c) Raw Sewage

Sewage samples have been analyzed previously using the ozone-UV technique. The high pressure oxygen procedure was much more effective, and yielded a clear liquid with inorganic sediment.

(d) Urine

Oxidation of the organic components of urine was only partially achieved, yielding a filterable liquid which required additional treatment. The ozone-UV method has been found effective for oxidation of urine, but it frequently yields a precipitate of calcium oxalate.

(e) Cellulose Esters (Millipore Filter)

A Millipore filter was dissolved in acetone and the mixture dispersed ultrasonically in water to give an emulsion of cellulose esters. The high pressure oxygen procedure was successful in oxidation of this emulsion, yielding a clear filterable liquid. The ozone-UV method was completely ineffective.

(f) Lake Water

Both the high pressure oxygen and ozone-UV methods were effective in oxidation of the organics present in lake water, although some more refractory organics are known to remain after the ozone-UV treatment.

For evaluation of the high pressure oxygen technique, an oscillating high pressure reactor which did not require a mechanical stirrer was selected, thus eliminating one source of possible contamination. The equipment is shown in Figure 9. Initial experiments were performed using the oscillaing high pressure oxygen reactor at conditions up to 300^{o}C and 13.8 MPa. The immediate conclusion of the first experiment was that glass inner containers cannot be used at temperatures above 200^{o}C, since they are attacked by water under these conditions. It is possible that other container materials may be more resistant, but the choices are somewhat restricted in view of the high pressure oxygen conditions. After a preliminary test using silica tubing, silica containers were rejected since a precipitate of silica was found after the treatment. A custom-designed Teflon^{R} container was eventually fabricated, and this was found to be satisfactory.

At the highest pressure and temperature used, namely 13.8 MPa and 300^{o}C, it was found that morphological changes occurred in chrysotile. Although no change in the Mg/Si ratio was found, non-fibrous debris was generated by the procedure and some of the fibers showed evidence of unscrolling.

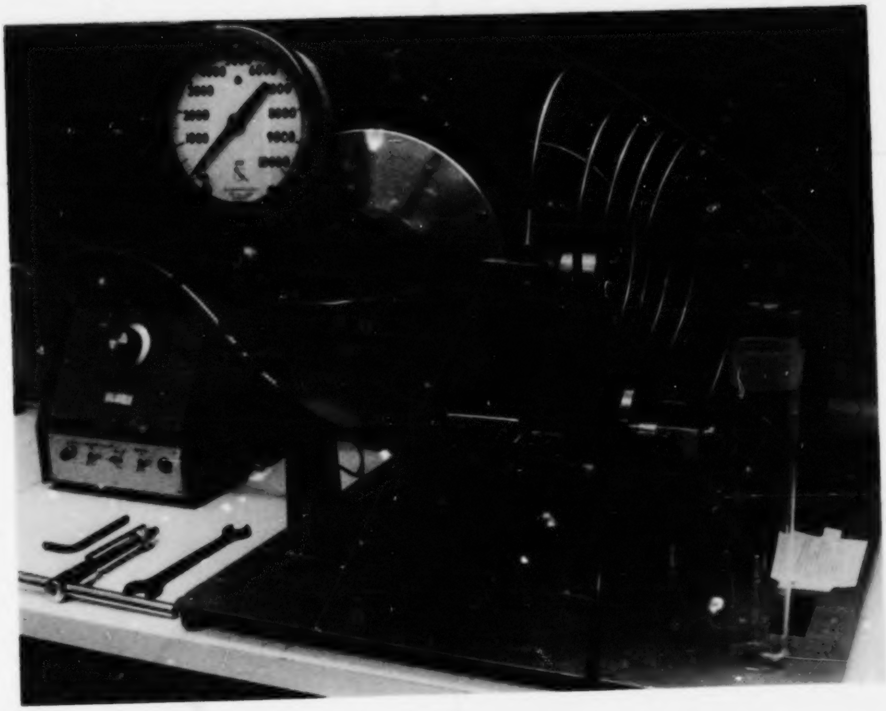

Figure 9. High pressure oxidation equipment.

Figures 10 and 11 show the comparison of the fiber morphologies before and after the treatment. The non-fibrous debris was found to contain magnesium and silicon, giving rise to speculation that the fibers had possibly reacted with the water under the extreme conditions. It is possible that reduction of the temperature and pressure would permit oxidation of the organics to be achieved without significant degradation of the fibers. Since the amphiboles are generally less reactive than chrysotile, it is likely that these minerals would be unaffected.

The high pressure oxygen procedure may be useful for very difficult samples such as effluents with high concentrations of refractory organic materials. However, it was concluded that the ozone-UV method was certainly the superior technique for routine drinking water analyses, particularly in view of later observations on scavenging by the sample container surfaces. Accordingly, no further investigation of the high pressure oxygen technique was undertaken.

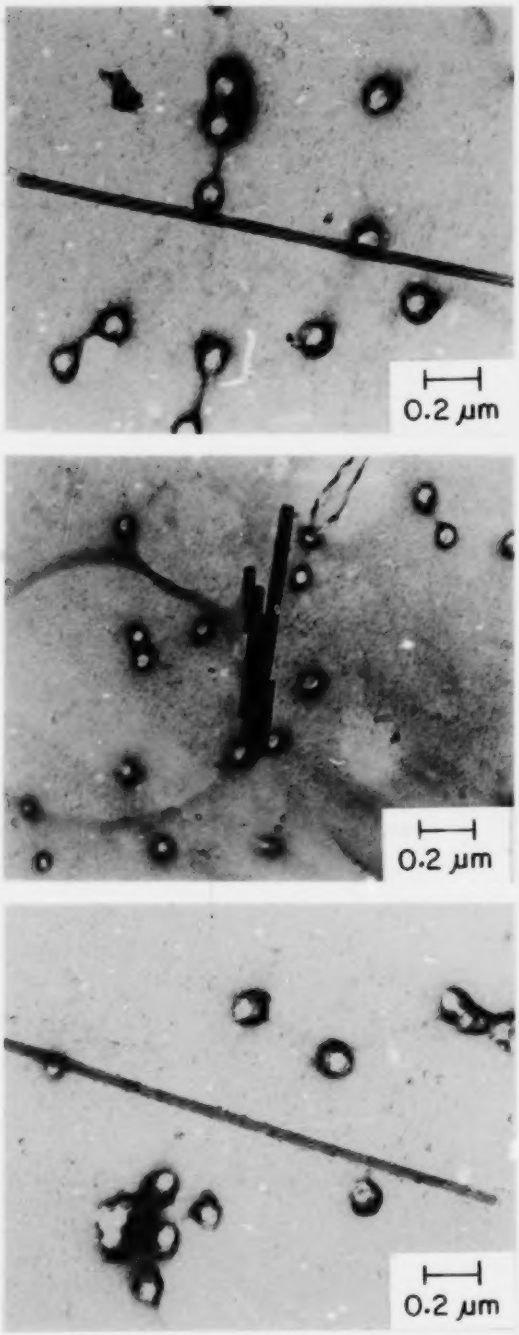
0.2 µm
0.2 µm
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Figure 10. Union Carbide Calidria Chrysotile before high pressure oxidation treatment.

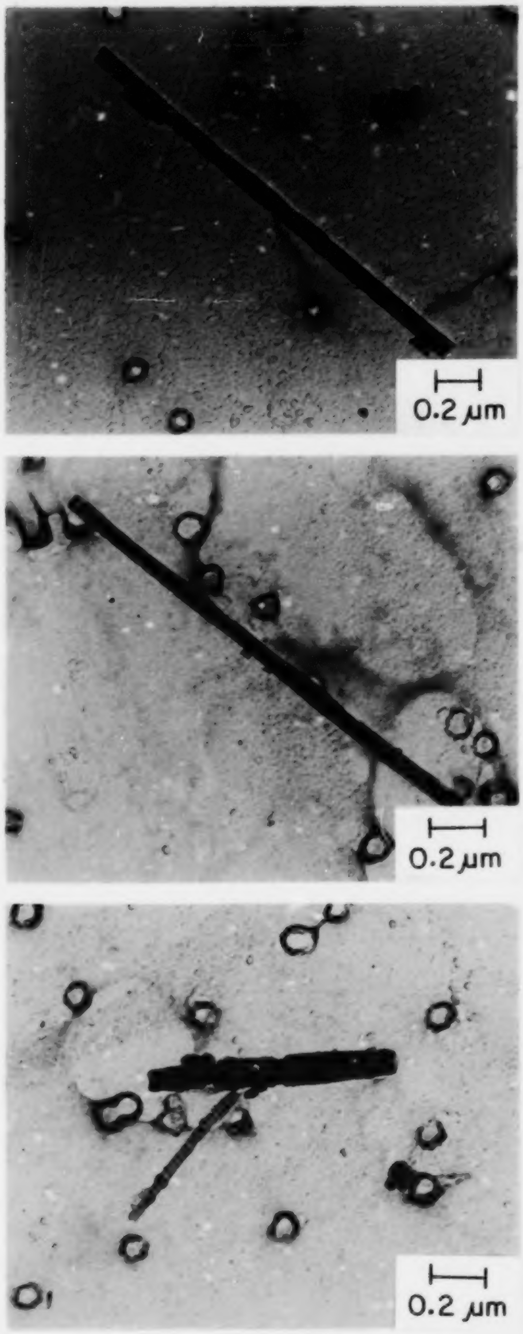
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Figure 11. Union Carbide Calidria Chrysotile after high pressure oxidation treatment.

SECTION 6

EXPERIMENTS ON THE STABILITY OF AQUEOUS FIBER DISPERSIONS

6.1 INITIAL OBSERVATIONS

It has been known for some time that aqueous dispersions of asbestos fibers can yield lower fiber counts when they are analyzed after storage for long periods of time. The reasons for this instability of the dispersions were unknown. The question arises as to why fiber dispersions apparently remain stable in a large water body, when samples collected from it and stored in bottles eventually become unstable. Reasons which have been put forward for the reduction in concentration with time range from complete dissolution of the fibers to "plating out" of fibers onto the interior surfaces of the container. Complete aqueous dissolution of silicate mineral fibers under normal temperature and pressure conditions was considered unlikely, and the mechanism of the suggested scavenging action by the container surfaces was not clear.

The stability of asbestos fiber dispersions in water was of interest from two points of view:

(a) it is important to know that the fiber concentration measurement obtained from a bottle of water is the same as that which would have been obtained at the time of collection; and

(b) in a reference analytical method, stable fiber dispersions of known concentrations are required for the purposes of analytical quality assurance and qualification of individual analytical laboratories.

Accordingly, investigations were made into the factors which control the stability of asbestos fiber dispersions in water.

It has been established that, within a single laboratory, replicate filters prepared from the same dispersion of a single fiber type can yield replicate results when analyzed using the carbon-coated Nuclepore procedure.[7] The results of Section 4 have shown that the same is also true for chrysotile and amphibole fiber dispersions of natural origin. Inter-laboratory analyses of filters, all prepared by one laboratory from a single fiber dispersion, have also yielded comparable results.[22] However, inter-laboratory analyses of liquid samples have not in general yielded satisfactory results.[6,11]

Figures 12 and 13 show the results obtained when glass ampoules containing dispersions of purified Union Carbide Calidria chrysotile at two different concentrations in double-distilled water were distributed to a number of laboratories experienced in TEM determination of asbestos fiber concentrations. It is clear that in addition to an unacceptable degree of inter-laboratory variability, there was also a significant trend towards lower concentrations when there were longer time intervals between issue of the liquid samples and preparation of the analytical filters. The results from the Ontario Research Foundation are identified as triangles, and show that after an extended period of storage repeat analyses using techniques identical in all respects did not always yield values comparable to the initial fiber counts. Figure 14 shows similar results for a naturally-occurring dispersion of chrysotile (Lloyd Lake, Ontario), and it can be seen that the results also show a trend towards lower values with increasing periods of storage.

A large number of these ampoules had been prepared, and consequently material was available for studies to determine the reasons for their failure as reference standards. Several ampoules were opened and the contents filtered directly without the use of any ultrasonic treatment. No chrysotile fibers were detected on these filters, indicating that the chrysotile was no longer in suspension. The empty ampoules were then cut open so that the interior surfaces could be gold coated and examined in the SEM. An interesting feature emerged from this examination: many chrysotile asbestos fibers were found attached to the glass surface, and *all of them* were associated with some organisms or organic material of a gelatinous appearance. This effect is illustrated in Figures 15 and 16. It was evident that the asbestos fibers were cemented to the interior surfaces of the glass ampoules by this organic material. The results of the inter-laboratory analyses indicate that ultrasonic treatment of the ampoules before they were opened was only partially successful in redispersing the fibers. The origin of the organic material in these samples was unclear at this point.

In an initial experiment, a chrysotile asbestos dispersion was divided into nine equal volumes of about 800 mL each in one-liter polyethylene bottles. Samples from three of these bottles were filtered immediately for analysis. The remaining six bottles were treated as follows:

(a) two bottles were shaken continuously in a reciprocal shaker;

(b) two bottles were exposed continuously to ultrasonic agitation in a bath; and

(c) two bottles were allowed to stand on the bench, followed by 15 minutes in an ultrasonic bath before sub-sampling the liquid from them.

One bottle from each treatment was removed for analysis after a period of 24 hours, and the second one after seven days. Sub-samples from each group of three bottles were then filtered and the filters were prepared for TEM fiber counting. The results obtained are shown in Table 7. It

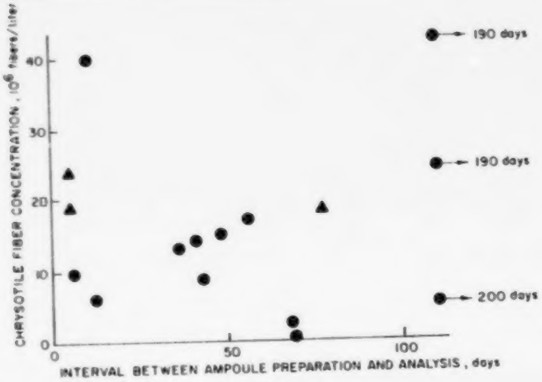


Figure 12. Results of inter-laboratory analyses using aqueous dispersion of Union Carbide chrysotile fibers (high concentration).

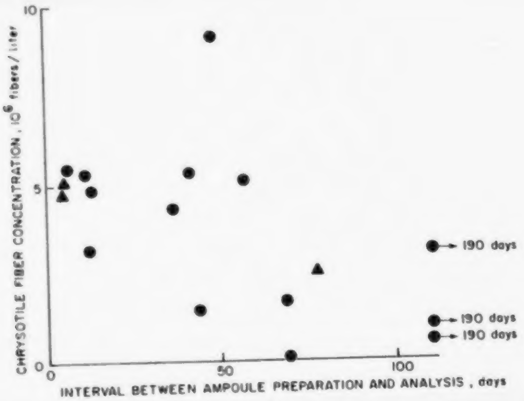


Figure 13. Results of inter-laboratory analyses using aqueous dispersion of Union Carbide chrysotile fibers (low concentration).

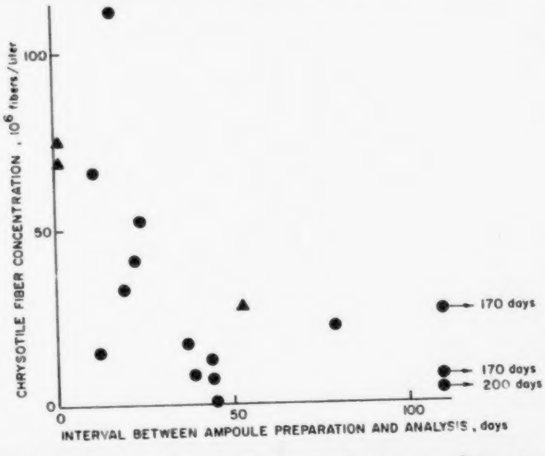
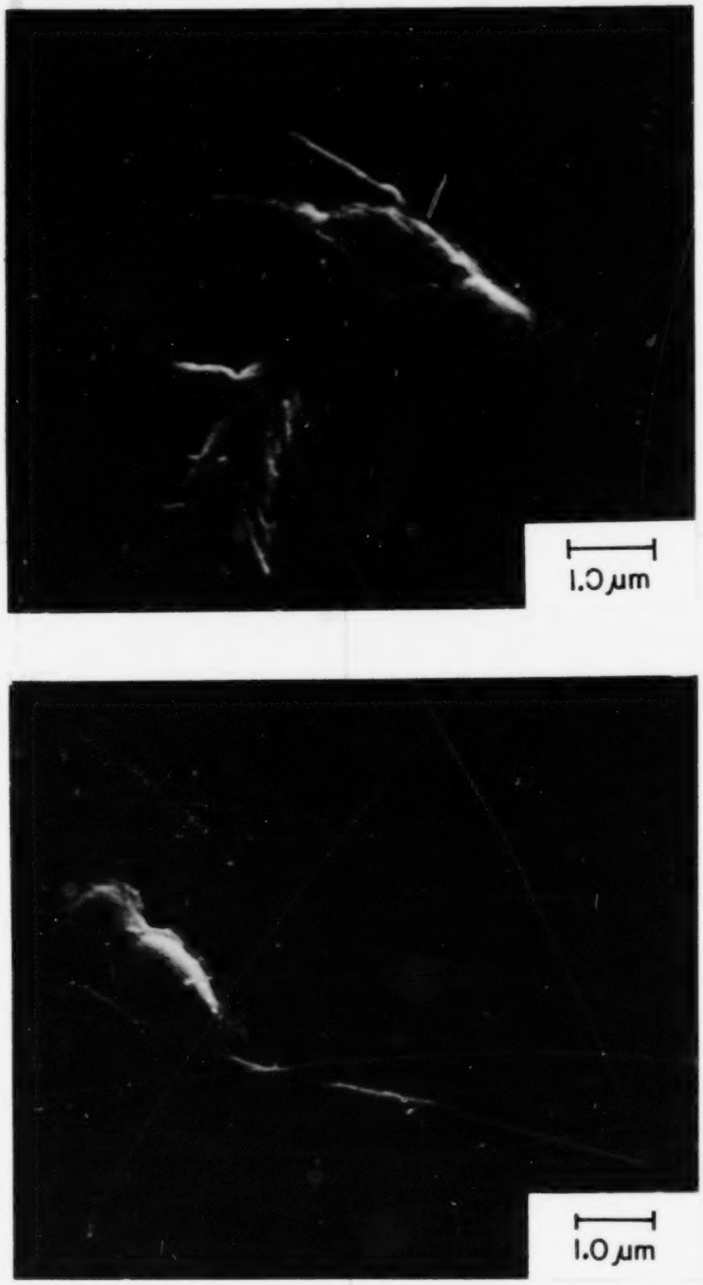


Figure 14. Results of inter-laboratory analyses using a naturally-occurring aqueous dispersion of chrysotile fibers. (Lloyd Lake, Ontario).



Figures 15 and 16. SEM micrographs showing asbestos fibers attached by organic material to the inside surface of a glass container.

is immediately evident that continuous shaking removed all of the fibers from suspension within 24 hours, whereas after seven days in an ultrasonic bath the results were essentially unchanged. In static storage, followed by ultrasonic treatment for 15 minutes prior to filtration, the concentration was reduced by about a factor of 2 after seven days. The curious and unexpected results for continuous shaking were thought to be due to collection of fibers by the inside surfaces of the containers, and it was considered that re-suspension might be achieved by ultrasonic treatment. The bottles, which had been continuously shaken for 24 hours and seven days respectively, were placed in an ultrasonic bath for a period of 30 minutes, and it was found that the re-suspended fiber concentrations were less than 10% of the initial value. This result was very disturbing, in view of the fact that the degree of agitation experienced by a water sample before it is analyzed cannot be controlled. A second experiment was conducted in which the fiber dispersions were prepared in a 0.005 Molar solution of sodium pyrophosphate ($Na_4P_2O_7$). This material is a recommended dispersal agent for silicate mineral particles.[24] The results obtained were essentially the same as those obtained without the sodium pyrophosphate: continuous shaking completely removed the fibers from suspension, whereas during static storage or continuous ultrasonic treatment the fibers mostly remained in suspension.

TABLE 7. STABILITY OF VERY DILUTE CHRYSOTILE FIBER DISPERSIONS

(Fiber concentrations in 10^6 fibers/liter)

Time	Treatment		
	Continuous Shaking	Continuous Ultrasonic	Static Storage with Routine Preparation
Initial	107	100	117
24 hours	<0.6	Not Analyzed	75
7 days	<0.6	78	51

Experiments were performed with polyethylene bottles containing equal volumes of the same dispersion, which contained approximately 3000 ng/liter of Union Carbide chrysotile.

In a separate and parallel study, experiments were conducted in order to assess the effects of pH and polyvalent ions on the stability of aqueous dispersions of chrysotile. Naumann and Dresher[25] reported that the stability of concentrated chrysotile dispersions is enhanced by addition of polyvalent ions to the dispersion at particular values of pH, and it was thought that the same technique might be useful at low fiber concentrations. The following ionic and pH conditions were used:

(a) aqueous dispersion adjusted to pH 4.0 using acetic acid;

(b) 0.005 molar ferric chloride adjusted to pH 4.0 using acetic acid; and

(c) 0.005 molar zirconyl chloride adjusted to pH 4.0 using acetic acid.

The results obtained are shown in Table 8, and were similar to those obtained previously: continuous shaking for 7 days removed most of the fibers from suspension, whereas during static storage for the same period most of the fibers remained in suspension. These results indicated that none of the wide range of conditions used appeared to affect significantly the final result: shaking of the suspension removed fibers almost completely, presumably to the interior surfaces of the plastic container.

TABLE 8. SHAKING OF CHRYSOTILE FIBER DISPERSIONS UNDER SELECTED IONIC AND pH CONDITIONS

(Fiber Concentrations in 10^6 fibers/liter)

Ionic and pH Conditions	Time	Treatment	
		Continuous Shaking	Static Storage with Routine Preparation
Aqueous Dispersion, Adjusted to pH 4.0 by addition of acetic acid	Initial	50	50
	7 days	0.6	37
0.005M $FeCl_3$, Adjusted to pH 4.0 by addition of acetic acid	Initial	41	41
	7 days	10	33
0.005M $ZrOCl_2$, Adjusted to pH 4.0 by addition of acetic acid	Initial	33	33
	7 days	2.6	20

6.2 EFFECTS OF BIOLOGICAL ORGANISMS ON SUSPENSION STABILITY

Throughout the work described in Section 6.1, no particular effort had been made to maintain sterility in the suspensions, and it was at this point that the very important part which bacteria and their decomposition products play in the stability of dilute dispersions of chrysotile asbestos fibers was recognized. The importance of the electro-kinetic effects of chrysotile in removal of polysaccharides is well known in the beverage and pharmaceutical industry,[26,27] and this lends support to the view that these organic materials may scavenge chrysotile fibers from suspension and subsequently adhere to the container walls.

New dispersions of Union Carbide chrysotile, UICC Canadian chrysotile and UICC crocidolite were prepared, using sterilized glass containers and double-distilled water taken directly from the condenser outlet of a glass still. The dispersions were immediately transferred to 50 mL glass ampoules, and these were flame sealed. The ampoules were then autoclaved at 121°C for 30 minutes in order to sterilize the contents. All of the preparation for each fiber type was completed within one day. Using this procedure, few bacteria or their decomposition products could be present in the dispersions, and any organisms which may have accumulated during handling would be destroyed, so that no biological activity should exist in the final ampoules. Table 9 shows the results obtained from analyses of 10 mL samples from the ampoules of Union Carbide chrysotile dispersion. It can be seen that even after storage for a period of sixty days, the fiber count remained unchanged, and that constant results were obtained without the use of ultrasonic treatment. The dispersions also showed no change after continuous shaking for periods of up to seven days. However, when an ampoule was opened and the contents contaminated with unsterile distilled water before being re-sealed, it was found that very variable fiber counting results could be obtained after shaking. However, the nature and concentration of the organisms added were not under good control. The results of similar work using UICC chrysotile are shown in Table 10. The sterile suspensions remained unchanged for long periods, but when they were contaminated with water containing active organisms the results were again unsatisfactory. In particular, the unsterile ampoule shaken for seven days yielded a very inhomogeneous filter, and the chrysotile fibers were found to be strongly aggregated and attached to organic debris. In this sample a large mass of organic material containing 20 fibers was found on one grid opening, resulting in a very large confidence interval for the calculated concentration value.

Although an extended study using crocidolite was not made, the results shown in Table 11 indicate that stable dispersions of UICC crocidolite can also be made if biological material is excluded from the preparation.

It has now become clear that much of the variability associated with interlaboratory analyses of aqueous dispersions of chrysotile fibers was due to the presence of biological organisms, the decomposition products of which scavenged the fibers and subsequently adhered to the surface of the containers. The fiber concentration measurement obtained was then a function of the extent to which this process had already occurred and the

TABLE 9. STABILITY AND STORAGE OF STERILE UNION CARBIDE CHRYSOTILE FIBER DISPERSIONS

(All concentration values in 10^6 fibers/liter)

Sample	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	82.6	44.5 - 121
After continuous shaking for 24 hours	73.5	47.1 - 99.9
After continuous shaking for 7 days	69.5	51.5 - 87.5
After 60 days storage, no ultrasonic treatment used	76.8 80.0	55.2 - 98.5 61.3 - 98.7
Unsterile ampoule shaken for 24 hours	29.4	15.0 - 43.8
Unsterile ampoule shaken for 7 days	16.7	4.4 - 29.0
Unsterile ampoule shaken for 4 days	62.4	38.8 - 86.4

TABLE 10. STABILITY AND STORAGE OF STERILE UICC CHRYSOTILE FIBER DISPERSIONS

(All concentration values in 10^6 fibers/liter)

Sample	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	19.1	11.1 - 27.0
After continuous shaking for 24 hours	27.9	18.2 - 37.6
After continuous shaking for 7 days	25.1	15.9 - 34.3
After 52 days storage, no ultrasonic treatment used	24.8 22.4	12.5 - 37.0 15.9 - 28.9
Unsterile ampoule shaken for 24 hours	29.4	21.0 - 37.8
Unsterile ampoule shaken for 7 days	38.8	0 - 77.7
Unsterile ampoule shaken for 4 days	6.4	3.3 - 9.6

TABLE 11. STABILITY AND STORAGE OF STERILE UICC CROCIDOLITE FIBER DISPERSIONS

(All concentration values in 10^6 fibers/liter)

Sample	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	28.9	15.4 - 42.4
After 30 days storage, no ultrasonic treatment used	34.7	26.9 - 42.5
	29.8	23.2 - 36.5

degree to which any ultrasonic treatment was able to detach and redisperse the fibers. If biological organisms were excluded, and absolute sterility was subsequently maintained, standard dispersions of chrysotile and crocidolite could be prepared which appeared to be stable for long periods of time. These observations on the effects of organic materials also indicate that the use of ultrasonic treatment on water samples prior to sub-sampling for analysis is absolutely essential if reliable and reproducible results are to be obtained.

6.3 PREPARATION AND PRESERVATION OF REFERENCE FIBER DISPERSIONS

Asbestos fiber dispersions were prepared and sealed in ampoules so that stable dispersions of known fiber concentration were available for the balance of the experimental program. Both flame-sealed borosilicate glass ampoules and Teflon^{R}-capped serum bottles are satisfactory containers in which sterility can be maintained. The procedure for preparation of the dispersions is described in Steps (a) to (e).

a) The weight of asbestos required to yield the desired mass concentration is calculated.

b) The asbestos is weighed accurately and dispersed in a small volume of freshly-distilled water by grinding it gently in an agate mortar and pestle.

c) The slurry is then dispersed in a large volume of freshly-distilled water in a glass container which has been sterilized by ozone-ultraviolet light treatment. The container is then sealed using a plastic stopper, and it is treated in an ultrasonic bath for a period of about 30 minutes.

d) The suspension is shaken vigorously, and then the required volumes are pipetted into washed ampoules or serum bottles.

e) The ampoules or bottles are sealed, labelled and autoclaved for 30 minutes at 121°C to sterilize the contents.

Several ampoules selected from the series, including the first and last prepared, should then be analyzed to obtain the precise numerical fiber concentration. These analyses should be conducted very carefully, and a minimum of about 200 fibers should be counted in each so that the size distribution can be defined accurately. It has been found in practice that for size distributions characteristic of water-borne asbestos, standards having particular numerical fiber concentrations can be prepared by assuming that UICC chrysotile and UICC crocidolite contain approximately 2×10^{14} fibers/g and 4×10^{12} fibers/g respectively.

6.4 CONTAINER AND STORAGE EFFECTS ON FIBER DISPERSIONS

The observation that laboratory-prepared aqueous dispersions of chrysotile were de-stabilized by small amounts of biological material, raised questions about the effects of container materials and storage on environmental samples collected in the field. Drinking water usually contains significant amounts of biological materials, and in view of the effects discussed in Section 6.3, it appears that the type of containers used and the storage conditions may have a profound effect on the fiber concentration results obtained from field samples.

Previous work[28] on the effects of containers and storage conditions on laboratory-prepared fiber dispersions in lake waters demonstrated that:

a) there was a large increase in the amount of skeletal diatomaceous material during storage in glass bottles in illuminated conditions;

b) samples stored in polyethylene bottles did not show a large increase in diatomaceous material;

c) after ultrasonic treatment of the bottle before subsampling, and using the analytical techniques of the time, no significant reduction in the suspended fiber concentrations was detected after storage of the samples for 8 months.

The ability to collect water samples from a source,and to transport them without significant changes to a laboratory for analysis, is fundamental to all existing analytical techniques for the determination of asbestos fiber concentrations. Therefore, a study was made in which both laboratory-prepared and naturally-occurring fiber dispersions were placed in different types of containers and transported under realistic conditions to the laboratory.

Concentrated sterile dispersions of Union Carbide Calidria chrysotile, UICC Canadian chrysotile, and UICC crocidolite were prepared in sealed glass ampoules so that they could be transported and used to prepare samples at the location of the naturally-occurring dispersion. The naturally-occurring fiber dispersion selected for this study was the drinking water of Sherbrooke, Quebec, which is known to contain a high concentration of chrysotile fibers. Four types of one-liter container were investigated; flint glass, borosilicate glass, polyethylene and polypropylene.

At the sampling site the ampoules of the sterile dispersions were opened, and diluted dispersions were prepared in the four types of container by adding 20 mL of the concentrate to 400 mL of double-distilled water. Nuclepore filter controls were prepared at the same time from samples of the sterile dispersions. Samples of the natural dispersion were collected directly from the faucet into the four types of container. Since it was thought that the fiber scavenging effect of the containers may be a function of fiber concentration, additional samples of the natural dispersion were also diluted 1:3 in double-distilled water before they were placed in the containers. Care was taken to ensure that changes in the fiber concentration of the source during sampling could not significantly affect the results of the study. Each sample was collected directly into the particular container, and a small volume withdrawn immediately for filtration through a 0.1 μm pore size Nuclepore filter. The Nuclepore filter controls were analyzed later to obtain the fiber concentrations of the dispersions before any transport or storage effects had occurred.

The results obtained for the Union Carbide chrysotile dispersion are shown in Table 12. It can be seen that after transport and storage for 3 days, followed by the usual treatment of fifteen minutes in an ultrasonic bath before sub-sampling for analysis, the results obtained were statistically identical with those obtained by analysis of the control filters made at the time of preparation of the samples. All of the types of container yielded the same satisfactory results.

The samples were then stored for another 36 days, after which they were shaken on a laboratory shaker for 2 days. Sub-samples were taken for analysis, and it was found that the suspended fiber concentrations in the plastic containers had been reduced to the detection limit of the measurements. This is a result consistent with the earlier work. The fiber concentrations remaining in suspension in the glass containers had been reduced to about 30% of the original values, indicating that the fiber scavenging mechanism of glass surfaces was less effective. It was decided that if, as suspected, the fibers were attached by organic material to the inside surfaces of the containers, ozone-UV treatment of the sample in the storage container, for about 4 hours, could oxidize the organic material and release the fibers into suspension. In Table 12 it can be seen that this was the case, and the most satisfactory result was obtained for the polyethylene container. Although the flint glass and polypropylene containers yielded results showing a similar pattern, the final results obtained were significantly lower than the fiber concentration of the original dispersion.

The results of the study using UICC chrysotile are shown in Table 13. The results obtained were similar to those using the Union Carbide

TABLE 12. STORAGE AND CONTAINER STUDY: UNION CARBIDE CHRYSOTILE

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material							
	Flint Glass		Polypropylene		Borosilicate Glass (Ground Glass Stopper)		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered at time of preparation of diluted samples in bottles	76.8	55.2 - 98.5	-	Not Analyzed	80.0	61.3 - 98.7	-	Not Analyzed
Storage for 3 days followed by 15 minute treatment in ultrasonic bath	83.3	61.1 - 106	80.4	63.8 - 97.0	85.3	66.2 - 104	78.5	54.0 - 103
Additional storage for 36 days followed by 48 hours on laboratory shaker. No further ultrasonic treatment	30.7	17.9 - 43.3	0.6	0 - 1.94	26.0	19.8 - 32.1	<0.5	-
Additional storage for 83 days followed by ozone-ultraviolet treatment and 15 minutes ultrasonic agitation in bath	49.7	27.6 - 71.7	40.7	25.5 - 55.8	-	Not Analyzed	65.0	55.0 - 74.5

*All concentration values refer to the fiber concentration in the original undiluted suspension. Analysis of the undiluted suspension at the time of the ampoule preparation yielded a concentration value of 82.6×10^6 fibers/liter, with a 95% confidence interval of $(44.5 - 121) \times 10^6$ fibers/liter.

TABLE 13. STORAGE AND CONTAINER STUDY: UICC CHRYSOTILE

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material							
	Flint Glass		Polypropylene		Borosilicate Glass (Ground Glass Stopper)		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered at time of preparation of diluted samples in bottles	24.8	12.5 - 37.0	-	Not Analyzed	22.4	15.9 - 28.9	-	Not Analyzed
Storage for 3 days followed by 15 minute treatment in ultrasonic bath	27.3	16.7 - 37.8	23.9	18.8 - 29.2	25.2	18.4 - 32.0	22.3	17.1 - 27.6
Additional storage for 56 days followed by 48 hours on laboratory shaker. No further ultrasonic treatment	7.7	2.9 - 12.5	4.5	0.6 - 8.3	5.0	0.1 - 9.9	<0.6	-
Additional storage for 89 days followed by ozone-ultraviolet treatment and 15 minutes ultrasonic agitation in bath	31.3	21.6 - 41.1	-	Not Analyzed	37.5	21.7 - 53.3	36.6	26.9 - 46.1

*All concentration values refer to the fiber concentration in the original undiluted suspension. Analysis of the undiluted suspension at the time of the ampoule preparation yielded a concentration value of 19.1 x 10^6 fibers/liter, with a 95% confidence interval of (11.2 - 27.0) x 10^6 fibers/liter.

chrysotile. Storage of the samples for 3 days, followed by ultrasonic treatment, yielded values which were statistically compatible with the original value for the dispersion. Long term storage, followed by shaking for 2 days, reduced the suspended fiber concentrations significantly. In the case of the polyethylene container the concentration was reduced to the detection limits of the measurement. In the flint glass, borosilicate glass and polyethylene containers, it was found that the ozone-UV treatment restored the suspended fiber concentrations to values which were statistically compatible with the original value for the dispersion.

It was originally thought that the fiber scavenging effect by the container surfaces would be specific to chrysotile, since fibers of this mineral carry a surface charge which is of an opposite sign from that carried by particles of most other mineral species. If this were the case the same behavior would not be expected for crocidolite. The results of the container study using UICC crocidolite are shown in Table 14, and it can be seen that the pattern is very similar to that obtained for chrysotile. The fiber scavenging effect of the container surfaces was very pronounced for the plastic containers, and the ozone-UV treatment was effective in restoring the fibers to suspension.

The fiber scavenging effect of the container surfaces was also demonstrated for Sherbrooke, Quebec, drinking water. Table 15 shows the results obtained for the first series of samples. The three Nuclepore filters prepared at the time of sample collection showed very consistent results of 64.6×10^6, 63.4×10^6, and 66.4×10^6 fibers/liter. After storage for 40 days the containers were shaken to re-disperse settled particulate, and a sample from each was analyzed. The results obtained were significantly lower than those from the control filters, and even the use of ultrasonic bath treatment in the normal preparation technique did not yield fiber counts higher than about 50% of the original values. Subsequent continuous shaking for 48 hours produced some additional increase in fiber concentration for the glass container, but reductions occurred for the two plastic containers. After storage for 15 more days the samples were given ozone-UV light treatment for 3 hours without removing them from the containers, followed by mild ultrasonic agitation in a bath for 15 minutes. After this treatment the fiber concentrations were restored to values statistically indistinguishable from those originally observed at the time of collection. These results were parallel to those obtained using laboratory-prepared dispersions in that shaking appeared to enhance the scavenging of fibers by the walls of the plastic containers. The fibers scavenged by the container walls could then be returned to aqueous suspension by ozone-UV treatment.

Results from the second series of samples of Sherbrooke drinking water are shown in Table 16. In this case the Sherbrooke water was diluted 1:3 with double-distilled water before placing in the containers for transport and storage. The pattern of results was similar to that of the first series, except that the scavenging effect was much more effective. The conclusion could be drawn that either the magnitude of the effect was dependent on fiber concentration, or that some agent in the double-distilled water enhanced it.

TABLE 14. STORAGE AND CONTAINER STUDY: UICC CROCIDOLITE

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material							
	Flint Glass		Polypropylene		Borosilicate Glass (Ground Glass Stopper)		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered at time of preparation of diluted samples in bottles	34.7	26.9 - 42.5	-	Not Analyzed	29.8	23.2 - 36.5	-	Not Analyzed
Storage for 3 days followed by 15 minute treatment in ultrasonic bath	31.7	24.2 - 39.3	30.9	22.7 - 39.1	15.4	10.7 - 20.2	35.3	27.3 - 43.3
Additional storage for 49 days followed by 48 hours on laboratory shaker. No further ultrasonic treatment	27.8	18.6 - 37.1	<1.1	-	4.4	0.2 - 8.6	0.7	0 - 2.3
Additional storage for 96 days followed by ozone-ultraviolet treatment and 15 minutes ultrasonic agitation in bath	36.5	26.7 - 46.3	-	Not Analyzed	30.6	19.7 - 41.5	25.2	13.4 - 37.0

*All concentration values refer to the fiber concentration in the original undiluted suspension. Analysis of the undiluted suspension at the time of the ampoule preparation yielded a concentration value of 28.9×10^6 fibers/liter, with a 95% confidence interval of $(15.4 - 42.4) \times 10^6$ fibers/liter.

TABLE 15. STORAGE AND CONTAINER STUDY: SHERBROOKE WATER (1st SERIES)

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material					
	Flint Glass		Polypropylene		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered on site	64.6	38.7 - 90.5	63.4	51.6 - 75.2	66.4	48.3 - 84.5
Storage for 40 days, hand shaken before sub-sample removed	15.3	11.3 - 19.3	10.4	8.4 - 12.5	22.5	15.0 - 29.9
Additional 15 minute treatment in ultrasonic bath	26.9	20.7 - 33.0	20.7	16.2 - 25.2	37.3	31.0 - 43.6
Additional 48 hours on laboratory shaker. No further ultrasonic treatment	35.5	31.1 - 40.0	15.2	12.2 - 18.3	26.9	20.8 - 33.0
Additional storage for 15 days, followed by ozone-ultraviolet treatment and agitation in ultrasonic bath for 15 minutes	73.2	57.9 - 88.4	73.0	62.0 - 84.0	62.9	36.6 - 89.1

*All concentration values in 10^6 fibers/liter refer to the fiber concentration in the original undiluted suspension.

TABLE 16. STORAGE AND CONTAINER STUDY: SHERBROOKE WATER (2nd SERIES)

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material					
	Flint Glass		Polypropylene		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered on site	65.0	47.7 - 82.3	42.4	31.9 - 52.8	49.1	33.9 - 64.3
Storage for 104 days, hand shaken before sub-sample removed	10.4	7.2 - 13.5	5.9	3.7 - 8.1	7.8	4.3 - 11.3
Additional 15 minute treatment in ultrasonic bath	25.3	21.0 - 29.7	21.3	16.8 - 25.7	18.1	14.1 - 22.1
Additional 48 hours on laboratory shaker. No further ultrasonic treatment	30.5	16.8 - 44.1	7.4	6.0 - 8.8	4.0	2.0 - 6.1
Additional storage for 3 days, followed by ozone-ultraviolet treatment and agitation in ultrasonic bath for 15 minutes	74.5	61.0 - 88.5	43.7	35.5 - 52	53.0	33.4 - 72.5

*All concentration values in 10^6 fibers/liter refer to the fiber concentration in the original undiluted suspension.

In the third series of Sherbrooke water samples no dilutions were made. The tests made on this series did not incorporate the extended mechanical shaking step, a procedure which would not normally be a component of routine sampling and analysis. The results are shown in Table 17. It can be seen that after storage for only 40 days, very low fiber concentration values were obtained when the samples were simply hand-shaken before sub-samples were removed. Treatment of the containers for 15 minutes in an ultrasonic bath elevated the fiber concentrations to about 30 - 50% of the value obtained at the time of collection. After storage for a further period of 41 days, the samples were treated with ozone-UV light and agitated for 15 minutes in an ultrasonic bath. The fiber concentrations were then found to be statistically indistinguishable from that obtained from the Nuclepore control filter prepared at the time of sample collection 81 days earlier.

The fiber length distributions at the various stages of the Sherbrooke water studies were found to be unchanged from those of the initial fiber dispersions determined from the Nuclepore filter controls. The tabulated fiber length distributions are shown in Appendix C.

The overall conclusions concerning container and storage effects were as follows.

a) Plastic containers scavenged more fibers than glass containers.

b) Samples stored for more than a few days yielded very low fiber concentration values.

c) Ultrasonic treatment by itself was not able to re-disperse all of the fibers scavenged by the container surfaces.

d) The ozone-UV technique, combined with ultrasonic treatment, was able to re-disperse the fibers scavenged by the container surfaces such that the suspended fiber concentration was restored to its original value at the time of collection.

e) Fibers from laboratory-prepared dispersions were scavenged more completely by the container surfaces than those from naturally-occurring dispersions.

f) The fiber scavenging effect did not appear to be size-selective.

6.5 INVESTIGATION OF FIBER SCAVENGING MECHANISM

Limited investigations were carried out in order to determine the agents responsible for the fiber scavenging by container surfaces. The effect was considered to be an important one, since it could potentially be developed into a separation technique specific for chrysotile asbestos fibers. Moreover, if the consequences of the effect are to be controlled in routine sample handling, a full understanding of the mechanism is required.

TABLE 17. STORAGE AND CONTAINER STUDY: SHERBROOKE WATER (3rd SERIES)

(All concentration values in 10^6 fibers/liter)*

Sample Treatment	Container Material					
	Flint Glass		Polypropylene		Polyethylene	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Water filtered on site	73.5	58.2 - 88.9	-	Not Analyzed	-	Not Analyzed
Storage for 40 days, no shaking before sampling by pipet	17.2	13.0 - 21.4	5.1	3.5 - 6.6	15.0	12.2 - 17.8
Additional 15 minute treatment in ultrasonic bath	39.9	30.7 - 49.1	15.8	12.3 - 19.3	25.7	17.2 - 34.2
Additional storage for 41 days, followed by ozone-ultraviolet treatment and agitation in ultrasonic bath for 15 minutes	68.6	38.0 - 99.2	94.3	53.4 - 135	62.8	60.8 - 64.8

*All concentration values in 10^6 fibers/liter refer to the fiber concentration in the original undiluted suspension.

The earlier observations on the stability of fiber dispersions indicated that the container itself did not appear to be responsible for the fiber scavenging effect, and that the presence of some biological agent or organic material was required. Moreover, it was found that plastic surfaces scavenged more fibers than glass surfaces. These observations are consistent with work by Fletcher[29] on bacterial attachment, in which it was found that the number of bacteria which became attached to surfaces immersed in water depends on the nature of the surfaces. In particular, it was found that much lower numbers of bacteria attached to surfaces such as glass or mica, than was the case for plastic surfaces such as polyethylene and polystyrene. It was also shown that RF plasma treatment of the plastic surfaces reduced the numbers of bacteria which attached to them. A possible mechanism for the fiber scavenging effect by sample container surfaces could be that the fibers become initially attached to the organic component or bacteria, which subsequently adheres to the container surface. Alternatively, it could be that the organic component coats the interior of the container such that fibers which contact the surface then adhere to it. In either case, mechanical agitation would assist the process by making contact with the container walls a more frequent event. Further experiments were conducted to elucidate the mechanism further.

The initial approach taken was to attempt identification of the organisms present in the non-sterile double-distilled water used for the container studies and in the stored Sherbrooke water samples. Standard plate counts were made to determine the total number of viable organisms present in the water samples. The double-distilled water was found to contain 2.1×10^7 organisms/liter and the Sherbrooke water 8.6×10^6 organisms/liter. Swabs taken from the inside walls of the containers also showed the presence of some fungi. The predominant viable organism in both water samples was Pseudomonas spp. Typical colonies of the micro-organisms were isolated and cultured in the laboratory. A washed suspension of the cultured micro-organisms was then employed in further fiber separation experiments.

The first series of experiments was designed to determine whether the cultured micro-organisms were effective in attachment of chrysotile fibers to the inside surfaces of polyethylene containers. About 800 mL of each water sample was placed in a one-liter polyethylene container, to which was added 50 mL of a sterile suspension of Union Carbide chrysotile. The container was then mechanically shaken for 60 hours at a frequency of about 2.5 Hz. The water was then transferred to an identical new polyethylene bottle, and given ozone-UV treatment in the usual manner, followed by exposure to ultrasonic agitation in a bath for 15 minutes. A sample was then filtered and analyzed. The original empty polyethylene bottle was re-filled with 850 mL of double-distilled water, and ozone-UV treatment was given to this sample, followed by 15 minutes in an ultrasonic bath. A sample was then filtered and analyzed. The results of the 6 experiments are shown in Table 18. It can be seen that the unfiltered, unsterile, double-distilled water (Experiment 1) yielded attachment of 84.5% of the chrysotile fibers to the container, a high value which is consistent with the earlier work. A second sample of the

TABLE 18. INITIAL FIBER SEPARATION EXPERIMENTS USING UNION CARBIDE CHRYSOTILE

Experiment	Fiber Concentration Remaining Suspended (10^6 fibers/liter)	Separated Fiber Concentration (10^6 fibers/liter)	Proportion of Fibers Separated, %
1. Unfiltered, unsterile double-distilled water	20.8	113	84.5
2. Unsterile double-distilled water, filtered through 0.2 μm Nuclepore filter	17.6	134	88.4
3. Unfiltered Mississauga tap water	100	39	28.1
4. Unfiltered Mississauga tap water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	61.7	45.5	42.4
5. Filtered Mississauga tap water	0.8	173	99.5
6. Filtered Mississauga tap water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	55.3	116	67.7

unsterile, double-distilled water was filtered through a 0.2 μm pore size Nuclepore filter before the experiment was performed, (Experiment 2). In this experiment 88.4% of the chrysotile fibers became attached to the polyethylene container, indicating that the active agent was capable of penetrating a 0.2 μm pore size filter. The experiment using unfiltered Mississauga tap water (Experiment 3), yielded an attachment of only 28.1%, which was not greatly increased in Experiment 4 by the initial addition of 10^7 viable micro-organisms/liter of the Pseudomonas spp culture. Filtered Mississauga tap water by itself (Experiment 5), yielded an almost complete attachment of 99.5% of the fibers, which appeared to be suppressed to 67.7% by initial addition of the cultured micro-organisms, (Experiment 6). The results, therefore, indicated that the active agent was present in Mississauga tap water, that it was capable of passing through a 0.2 μm pore size filter, and that the cultured micro-organisms appeared to be ineffective.

A second series of experiments was performed using the same procedure, firstly as a partial repeat of the first study, and secondly to extend it to UICC crocidolite and UICC amosite dispersions. The results for Union Carbide chrysotile are shown in Table 19. In Experiment 1, a high attachment of >99.6% was demonstrated using unsterile, double-distilled water. In Experiment 2, sterile conditions were maintained as efficiently as possible, using water directly from the output of the still. In this case only 5.9% of the fibers were observed to attach to the container walls. In Experiment 3, the sterile, double-distilled water was used again, but with the addition of 10^7 viable micro-organisms/liter of the culture. No significant attachment of fibers was observed as a consequence of the addition. In Experiment 4, addition of the cultured micro-organisms was made to Mississauga tap water, and the attachment of 75.5% agreed with that observed in the initial experiment (67.7%). Although the actual proportions of attached fibers were generally lower, the overall patterns of the results for crocidolite and amosite were similar to that observed for chrysotile. These results are shown in Tables 20 and 21.

In summary, the attachment of asbestos fibers to polyethylene surfaces appears to be promoted by an organic material produced by bacteria, rather than by the bacteria themselves. The material either coats the inside surface of the container, which then retains any fiber which contacts the surface, or it attaches to the fibers themselves which subsequently contact the container surface and remain there. The precise nature of the organic material is not clear, but it is likely to consist of some varieties of polysaccharide of bacterial origin.[30] These are generally water-soluble, protein free, relatively heat-resistant complexes with molecular weights up to 10^6. These materials are known to be present in drinking water,[31] and it has been shown that they are strongly adsorbed by chrysotile asbestos as a consequence of its very high positive zeta potential.[32] The action of these organic materials on the amphibole asbestos varieties is not currently understood. Further investigations were not made into the mechanism, but it is probable that the effect could be developed into a reliable, and at least partially-specific, separation technique for chrysotile asbestos. Application of the technique to the amphiboles is

TABLE 19. SEPARATION EXPERIMENTS USING UNION CARBIDE CHRYSOTILE

Experiment	Fiber Concentration Remaining Suspended (10^6 fibers/liter)	Separated Fiber Concentration (10^6 fibers/liter)	Proportion of Fibers Separated, %
1. Unfiltered, unsterile double-distilled water	< 0.5	129	> 99.6
2. Sterile double-distilled water	136	8.50	5.9
3. Sterile double-distilled water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	119	6.66	5.3
4. Unfiltered Mississauga tap water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	24.6	75.9	75.5

TABLE 20. SEPARATION EXPERIMENTS USING UICC CROCIDOLITE

Experiment	Fiber Concentration Remaining Suspended (10^6 fibers/liter)	Separated Fiber Concentration (10^6 fibers/liter)	Proportion of Fibers Separated, %
1. Unfiltered, unsterile double-distilled water	46.9	118	71.6
2. Sterile double-distilled water	189	1.85	1.0
3. Sterile double-distilled water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	136	8.63	6.0
4. Unfiltered Mississauga tap water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	58.4	79.1	57.5

less certain, and further understanding of the mechanism is required so that a controlled procedure can be developed.

TABLE 21. SEPARATION EXPERIMENTS USING UICC AMOSITE

Experiment	Fiber Concentration Remaining Suspended (10^6 fibers/liter)	Separated Fiber Concentration (10^6 fibers/liter)	Proportion of Fibers Separated, %
1. Unfiltered, unsterile double-distilled water	2.01	12.1	85.8
2. Sterile double-distilled water	55.6	1.73	3.0
3. Sterile double-distilled water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	46.6	4.37	8.6
4. Unfiltered Mississauga tap water with addition of 10^7 viable organisms/liter of Pseudomonas spp.	25.1	10.5	29.5

SECTION 7

FIBER IDENTIFICATION PROCEDURES

7.1 GENERAL CONSIDERATIONS

Precise methods for identification of asbestos fibers have never been specified as part of the published analytical procedures.[2,33] Consequently, the electron microscopist has been free to decide what combination of measurements or observations constitutes an adequate identification for a particular fiber. Chrysotile was originally thought to be relatively simple to identify, since similar tubular morphology occurs in only a few other minerals and its selected area electron diffraction (SAED) pattern has some characteristic features. However, it has been found that other species such as vermiculite can yield particles which have a scrolled structure, and these can be mistaken for chrysotile if the SAED patterns are not interpreted quantitatively.[34] It has always been considered difficult to identify the precise mineral species of a single submicrometer fiber of amphibole. The layer type SAED patterns which are usually obtained have a 0.53 nm layer separation. Comparison of this type of pattern with those obtained from known amphibole asbestos fibers has often been accepted as identification for an amphibole fiber, but in fact many other minerals can yield similar or identical patterns. Accordingly, the more cautious analyst has obtained energy dispersive X-ray analyses (EDXA) of at least a few of the fibers to provide additional confidence in the identification, although these analyses were not called for in the specified procedure. Even this approach is subject to error, since other minerals exist which have elemental compositions similar to those of the amphiboles. Therefore, as part of the analytical procedures for determination of asbestos fiber concentrations in water, a logical and practical protocol for fiber identification must be defined.

When the mineralogical species of the fibers has been correctly identified the question still remains as to whether the fibers are actually *asbestos*. Although chrysotile presents little difficulty in this regard, it is not routinely possible in the TEM to classify an individual small amphibole fiber as either asbestos or as a non-asbestos cleavage fragment, since crystal habit is the only basis on which these can be discriminated. Previous work[35] has shown that *populations* of fibers which originate from the fibrous and non-fibrous minerals can be discriminated on the basis of the distributions of their fiber aspect ratios. A demonstrated and reliable means of discrimination is necessary, since many minerals other than the asbestoses, but compositionally similar to them, yield fragments of relatively high aspect ratios. This aspect of fiber identification is discussed more fully in Section 8.

Before it is incorporated into a fiber count, each particle with an aspect ratio of 3:1 or greater, and not of obviously biological origin, must be identified according to precisely defined criteria. Fiber internal morphology, chemical composition and crystal structure are the properties on which these criteria must be based. Fiber internal morphology allows amphiboles and other crystalline mineral fibers to be discriminated from chrysotile and a few other minerals which display a tubular appearance in the TEM. Further analysis of each fiber must then be conducted using SAED and EDXA methods.

The crystal structure of some mineral fibers, such as chrysotile, is easily degraded by the high current densities required for EDXA examination. Therefore, SAED investigation of these sensitive fibers must be completed before attempts are made to obtain EDXA spectra. When examining more stable fibers, such as the amphiboles, the order of work is unimportant.

7.2 FIBER IDENTIFICATION TECHNIQUES

7.2.1 SAED Technique

The SAED technique can be either qualitative or quantitative. For quantitative work, a thin film of gold should be evaporated on the underside of the specimen grid as an internal calibration of camera length. Qualitative SAED consists of visual examination of the pattern obtained on the microscope screen from a randomly oriented fiber. For non-cylindrical fibers, quantitative (zone axis) SAED requires alignment of the fiber so that a principal crystallographic axis is parallel to the electron beam. The pattern is then recorded and its consistency with zone axis patterns from known mineral structures can be examined. The SAED pattern obtained from one zone axis may not be sufficiently specific to identify the mineral fiber, but it is often possible to tilt the fiber to another angle and to record a different zone axis pattern. The angle between the two axes can also be checked for consistency with the structure of a suspected mineral. Interpretation of the zone axis patterns, and computation of the interzone axial angles of known mineral structures are complex and are best performed by a computer program. SAED patterns obtained from fibers with cylindrical symmetry, such as chrysotile, are an exception since they are not sensitive to axial tilt, and patterns from randomly oriented fibers can be interpreted quantitatively.

For visual examination of the SAED pattern, the camera length of the TEM should be set to a low value and the SAED pattern then should be viewed through the binoculars. This procedure minimizes the irradiation and possible degradation of the fiber. However, the pattern is distorted by the tilt angle of the viewing screen. For recording purposes, a camera length of at least 2 meters must be used if accurate measurement of the pattern is to be possible. It is of extreme importance that, when obtaining an SAED pattern for either recording or visual evaluation, the sample height should be properly adjusted to the eucentric point and the image should be focussed in the plane of the selected area aperture. If this is not done there may be some components of the SAED pattern which do not originate from the selected area. It will be found in general that the smallest SAED aperture will be necessary.

If a zone axis SAED analysis is to be attempted on a fiber, the sample must be placed in an appropriate holder. The most convenient holder allows complete rotation of the sample and single axis tilting. The sample should be rotated until the fiber image indicates that the fiber is oriented with its length coincident with the tilt axis of the goniometer. The sample height should then be adjusted until the fiber is at the eucentric position. The fiber is tilted until a pattern appears which is a symmetrical, two-dimensional array of spots. The recognition of zone axis alignment conditions requires some experience on the part of the operator. Not all zone axis patterns which can be obtained are useful or definitive. Only those which have closely-spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0.3 nm are not specific and are usually very wasteful in computer analysis time. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between the reflections are usually the most definitive.

7.2.2 EDXA Technique

Correct identification of individual mineral fibers requires quantitative data on their compositions. In addition, the quantitative analysis procedure should be transferable between instruments.

The technique described by Cliff and Lorimer[36] offers a convenient method by which relatively accurate (∿10%) quantitative analyses can be obtained. The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus the mass absorption and fluorescence effects are negligible. In a specimen composed of elements I and J, the following relationship can be used to perform quantitative analyses in the TEM.

$$\frac{A_I}{A_J} = k \frac{C_I}{C_J}$$

where A_I and A_J are the elemental integrated peak areas measured on the TEM, C_I and C_J are the weight or atomic fractions of the two elements, and k is a constant. To incorporate correction for the particle size effect on peak area ratios,[37] the Cliff and Lorimer technique has been extended by obtaining separate values of k for different ranges of fiber diameter.

Calibration of the TEM-EDXA combination was achieved using reference silicate minerals. Great care was exercised in the selection of suitable calibration standards, so that they were as homogeneous as possible. Since many of the fiber analyses will involve the commercial asbestos varieties, some of the standards were selected to have compositions close to these. Each mineral standard was examined under a binocular microscope, and a single crystal was extracted using forceps. The crystal was cleaved, and one fragment was embedded in methacrylate, after which it was polished for microprobe analysis. The other fragment was ground using a mortar and pestle. The crushed mineral was dispersed in water, which was then filtered using a 0.1 µm pore size Nuclepore filter. TEM grids were prepared from the filter using the carbon-coated Nuclepore procedure. EDXA spectra were obtained from about 20 fibers in each of four fiber diameter ranges. The spectra were corrected for background, and the peak area for each element was expressed as a ratio to the silicon peak area. Quantitative microprobe analysis was performed on the polished mineral fragment, and the composition of the mineral was determined using a conventional ZAF procedure. The mean k values obtained for a range of minerals are shown in Table 22 and these form the basis of the quantitative TEM-EDXA calculation procedure. If quantitative determinations are required for minerals containing elements other than those shown in Table 22, suitable standards can be selected and calibration information obtained by following the procedure described above.

The quantitative EDXA technique can readily be transferred to another system with only a minimum of calibration. Standards of some selected minerals in Table 22 have been prepared on TEM grids for this purpose, and the only requirement is to obtain EDXA spectra from about 20 particles of each mineral. The mineral standards selected for this purpose and the elements for which they are used are shown in Table 23.

TABLE 23. SILICATE MINERAL STANDARDS FOR CALIBRATION OF TEM-EDXA SYSTEM

Mineral Standards	Elements
Riebeckite	Na, Fe, Si
Chrysotile	Mg, Si
Halloysite	Al, Si
Phlogopite	K, Si
Wollastonite	Ca, Si
Bustamite	Mn, Si

TABLE 22. "K" VALUES

(Atomic Ratio/Peak Area Ratio, Relative to Silicon)

Element	Mineral	Fiber Width Range, μm			
		<0.25	0.25 - 0.5	0.5 - 1.0	>1.0
Mg	Actinolite	1.783	1.743	1.724	1.808
	Cummingtonite	1.673	1.651	1.712	1.746
	Grunerite	1.894	2.006	2.021	2.167
	Hornblende	1.741	1.687	1.770	1.943
	Talc	1.902	1.892	1.807	1.784
	Tremolite (A)	1.619	1.709	1.679	1.745
	Tremolite (B)	1.681	1.672	1.714	1.818
	Chrysotile	1.678	1.855	1.641	1.591
Fe	Actinolite	0.995	1.181	1.050	0.945
	Crocidolite	1.123	1.059	1.038	1.042
	Cummingtonite	1.160	1.189	1.136	1.046
	Grunerite	1.166	1.101	1.068	0.992
	Hornblende	1.043	1.021	0.956	0.885
	Phlogopite	N.A.	1.128	1.024	0.990
	Riebeckite	1.225	1.194	1.112	1.079
	Tremolite (A)	0.984	1.096	1.026	0.910
Ca	Actinolite	0.975	0.956	0.928	0.894
	Hornblende	1.001	0.971	0.935	0.867
	Tremolite (A)	0.870	0.853	0.836	0.814
	Tremolite (B)	0.801	0.901	0.824	0.777
	Wollastonite	1.080	1.050	1.036	0.993
Na	Crocidolite	2.979	3.185	3.324	3.475
	Riebeckite	3.344	3.177	3.631	3.890
K	Phlogopite	N.A.	1.026	0.925	0.916
Al	Halloysite	1.219	1.235	1.235	1.235
Mn	Bustamite	<— 1.198 —>		<— 1.181 —>	
	Rhodonite	<— 1.086 —>		N.A.	N.A.

7.2.3 Optimum Fiber Identification Procedure

Since heat-sensitive fibers such as chrysotile are degraded by electron beam irradiation, it is recommended that attempts to obtain SAED patterns be made first. The process of accumulating an EDXA spectrum requires intense irradiation of the fiber, and sensitive fibers will not always retain their crystallinity afterwards. However, interpretation of the fiber identification data is normally achieved using a computer program. It has been found that the most expedient identification procedure is first to select, on the basis of chemical composition, those minerals consistent with the EDXA spectrum of the unknown fiber. The more labor-intensive technique of quantitative interpretation of zone axis SAED patterns can then be used for comparison with a limited number of possible minerals, rather than with the entire mineral vocabulary.

7.3 INSTRUMENTAL LIMITATIONS

A modern analytical electron microscope (AEM) has a resolution of the order of 0.2 nm, which is adequate for imaging of the smallest fibers of interest. However, in using the instrument an appreciation of the analytical limitations is required.

The smallest area of the image from which an SAED pattern can be obtained is obviously a critical factor in the analyses, since it may be impossible in a heavily-loaded specimen to completely isolate a particular fiber under investigation. The smallest area which can be analyzed without interference from surrounding particles is given by:

$$A = \frac{\pi}{4}\left(\frac{D}{M} + 2000\ C_s\theta^3\right)^2$$

where:

A = Effective SAED area in μm^2

D = Diameter of SAED aperture in μm

M = Magnification of objective lens

C_s = Objective lens spherical aberration coefficient in mm

θ = Maximum required Bragg angle in radians

Although almost all instruments of current manufacture meet these requirements, many older instruments which are still in service do not. It is obviously not possible to reduce the area of analysis indefinitely by use of apertures smaller in diameter than those specified by the manufacturer, since there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens. It is extremely important that before SAED operation is attempted, the image should be properly focussed, since the analyzed area expands rapidly with the degree of de-focus.

For EDXA measurements, the AEM should have an illumination and condenser lens system which is capable of forming electron probes 100 nm or less in diameter. The minimum probe diameter possible is limited by the spherical aberration of the condenser lens system, and in some instruments the minimum beam diameter may not have adequate current density for the X-rays generated from very thin fibers to be detected. In some instruments, this problem is overcome by performing all EDXA measurements in scanning transmission electron microscopy mode (STEM).

It is not always possible to collect SAED and EDXA data from a particular fiber. It may be impossible to obtain a satisfactory SAED pattern if the fiber is too close to a bar of the support grid. When the sample is tilted to locate a zone axis, the grid bar may obscure the fiber. The fiber may also be inappropriately tilted about an axis perpendicular to the goniometer tilt axis so that no principal zone axes are encountered within the range of the goniometer. A double tilt goniometer allows precise alignment of a fiber zone axis, but in this type of goniometer it is not possible to align the fiber axis with one of the tilt axes. If the fiber is large, extensive twinning in the structure may prevent the observation of a simple zone axis pattern. In the case of EDXA, the fiber may be too close to a bar of the support grid, giving rise to a high background signal unrelated to that from the fiber. In other cases, the grid bars or particulate on the sample may shield the detector and no spectrum from the fiber will be obtained.

7.4 SPECIFICATION OF ADEQUATE INSTRUMENTAL PERFORMANCE FOR EDXA MEASUREMENT

The microscope specifications required for SAED performance have already been stated in 7.3; the EDXA performance is a function of the microscope, the EDXA crystal itself, and a number of geometrical factors. The minimum acceptable performance for X-ray analysis is therefore rather more difficult to specify. Since suitable standard samples are not available, evaluation of the combined TEM-EDXA system must be based on examination of the spectrum obtained from a small diameter reference fiber which is excited by an electron beam of known diameter. It was concluded that the specification should be stated in terms of the relevant X-ray line which is most difficult to measure. X-ray detectors are generally least sensitive in the low energy region, and so measurement of sodium in UICC crocidolite was selected as the performance criterion.

Investigations were made into the performance of the Philips EM400T and EDAX PV9760/04 30 mm^2 detector, which is considered to be a state-of-the-art instrumental combination for this type of analysis. An electron beam diameter of 100 nm, and an accelerating potential of 100 kV were selected. The beam was allowed to impinge on a UICC crocidolite fiber of 50 nm diameter, and it was found that a net NaKα peak area count rate of 5 cps was obtained, with a peak/background ratio of 5.0. Using an earlier model Philips EM301S and a Kevex 3214-301-V 10 mm^2 detector, a corresponding NaKα peak area count rate of 1 cps was obtained with a peak/background ratio of 1.0. It was considered that the net peak area should exceed 100 counts in order for it to be considered statistically valid. Accordingly, under these conditions using the EM301S, a counting period of

100 seconds would be required in order to accumulate a satisfactory spectrum. It has not been possible to survey all of the instrumental combinations in use, but the results of the EM301S - Kevex 3214-301-V combination represents a reasonable minimum performance which is acceptable. Relaxation of this performance requirement leads to long counting times and perhaps even an inability to detect the presence of sodium in the smaller fibers normally found in water samples. Inability to detect sodium would seriously compromise the specificity of analysis, and in particular would not allow discrimination of crocidolite from some other mineral species. If the instrumental combination yields the specified performance for the NaKα line from typical UICC crocidolite fibers, the performance for the higher energy X-ray lines will normally exceed the requirements.

In some circumstances where detection of sodium is not important, for example in chrysotile analyses, the instrumental requirements could be relaxed and replaced by a similar criterion based on detection of magnesium in reference chrysotile fibrils.

7.5 ANALYSIS OF FIBER IDENTIFICATION DATA

Because the fiber identification procedure is involved and time-consuming, a Fortran computer program has been developed which permits the work to be performed by individuals without extensive training in mineralogy. The program is listed in the publication, Analytical Method for Determination of Asbestos Fibers in Water.[17] This program permits the EDXA and zone axis SAED measurements to be compared against a library of compositional and structural data for 226 minerals. The mineral library includes fibrous species which have been listed by several authors,[38-43] together with other minerals which are known to be similar to amphibole or chrysotile in either their compositions or some aspects of their crystallography. Amphibole compositional ranges and terminology were adopted in accordance with the most recent mineralogical classification by the International Mineralogical Association.[44] An example of a single mineral entry in the file is shown in Table 24.

TABLE 24. AN EXAMPLE OF A SINGLE MINERAL ENTRY IN THE MINERAL LIBRARY FILE

```
NAME:        CUMMINGTONITE
FORMULA:     (FE,MG)7 SI8 022 (OH)2
MANDATORY:      14  8.00  8.00  12  2.10  4.90   26   2.10  4.90    0   0.00  0.00
OPTIONAL:       20  0.00  1.34  11  0.00  1.34    0   0.00  0.00    0   0.00  0.00
A,B,C,ALPHA,BETA,GAMMA,SYM:        9.600  18.300     5.300  90.000 101.833   90.000     5
```

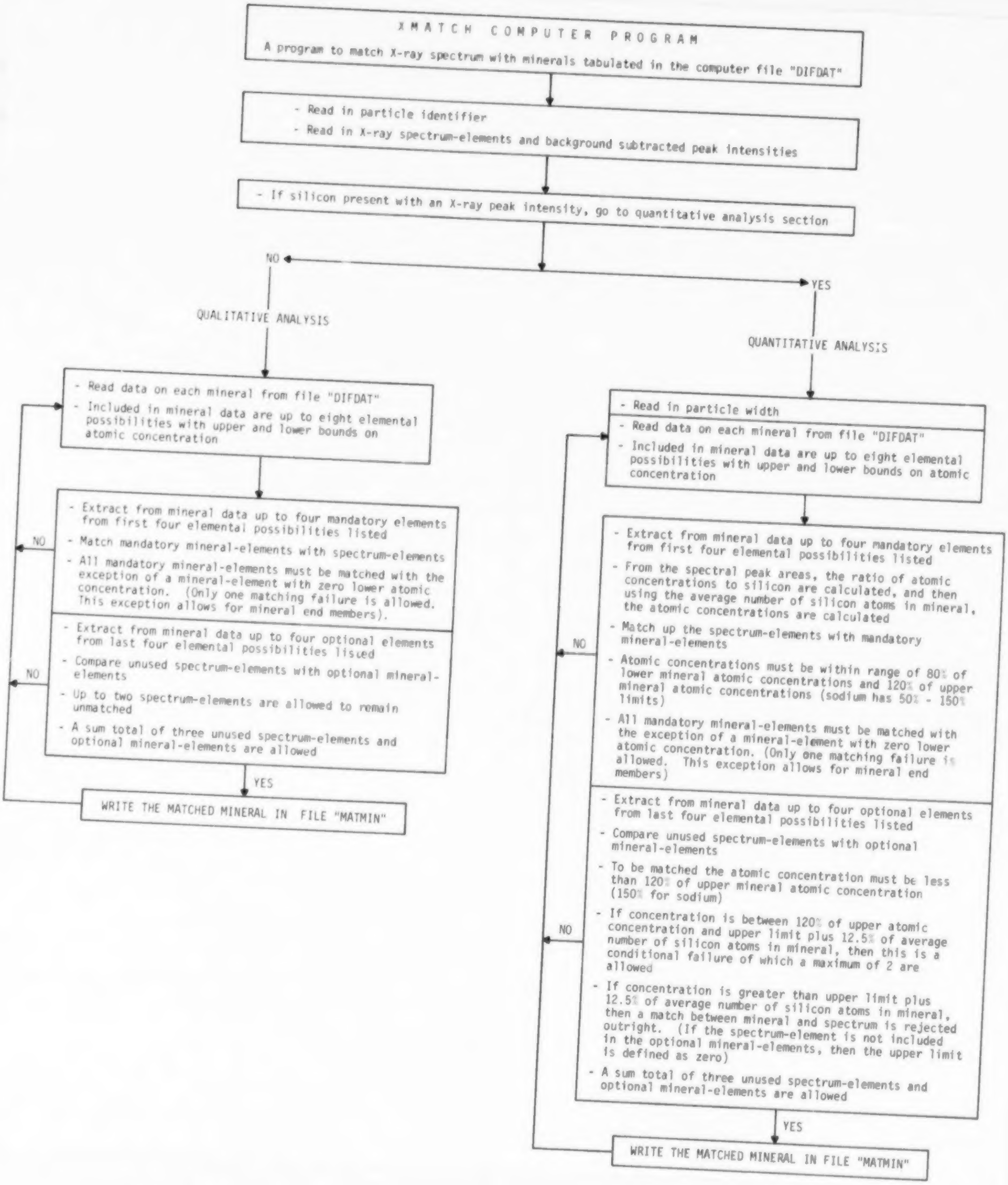
The nominal composition for the mineral is stated, along with the permitted ranges of the number of atoms for four mandatory elements. In the case of cummingtonite, the published ranges for the mandatory elements are Si (8.00), Mg (2.10 - 4.90), and Fe (2.10 - 4.90). Elements which may also be present, but which are not mandatory in this mineral, are Ca (0 - 1.34) and Na (0 - 1.34). The crystallographic data are also listed together with a numerical code for the symmetry group. Additional minerals may be added to the library if they are thought to be of concern in particular situations. The addition of new minerals to the data file does not require any further calibration of the EDXA system.

It is important to recognize that a demonstration that the measurements on an unknown fiber are consistent with the data for a particular comparison mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, very unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fiber identified uniquely by a quantitative chemical analysis and indexed SAED patterns from two zone axes.

The computer program classifies fibers initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used. The procedure using qualitative EDXA consists of entering the list of elements which originate from the particle. For quantitative EDXA, the list of elements and the areas under the corresponding X-ray emission peaks, after background correction, form the input data for the computer program. The calculated elemental composition of the unknown is compared with each mineral in the stored mineral library. The program selects from the file a list of minerals which are consistent in composition with that measured for the unknown fiber. The published compositional ranges specified in the data file are increased by ± 20% to accommodate experimental error. In the case of sodium, the ranges are increased by ± 50%, reflecting the increased difficulty of quantifying this element. For a mineral to be selected as consistent in composition, the mandatory elements must be present in the unknown and, if the input data is quantitative, then the mandatory elements must be present within these bounds. In addition, the remaining elements entered for the unknown fiber are compared either qualitatively or quantitatively with the optional elements in the comparison mineral.

Figure 17 shows a complete flow diagram for the matching procedure based on chemical composition. Figure 18 shows an example of the output obtained for a fiber of riebeckite. It can be seen that the original list of 226 minerals has been reduced to four compositionally-consistent varieties.

The computer program is linked to an electron diffraction pattern analysis routine which is an extension of that originally developed by Rhoades[45]. The program requires measurements of 5 reflections from the zone axis SAED pattern, which are then tested for consistency with the crystallographic data of the minerals previously selected as compositionally-consistent with the unknown fiber. If more than a single



A program to match X-ray spectrum with minerals tabulated in the computer file "DIFDAT"

- Read in particle identifier
- Read in X-ray spectrum-elements and background subtracted peak intensities

- If silicon present with an X-ray peak intensity, go to quantitative analysis section

- Read data on each mineral from file "DIFDAT"
- Included in mineral data are up to eight elemental possibilities with upper and lower bounds on atomic concentration

- Extract from mineral data up to four mandatory elements from first four elemental possibilities listed
- Match mandatory mineral-elements with spectrum-elements
- All mandatory mineral-elements must be matched with the exception of a mineral-element with zero lower atomic concentration. (Only one matching failure is allowed. This exception allows for mineral end members).

- Extract from mineral data up to four optional elements from last four elemental possibilities listed
- Compare unused spectrum-elements with optional mineral-elements
- Up to two spectrum-elements are allowed to remain unmatched
- A sum total of three unused spectrum-elements and optional mineral-elements are allowed

WRITE THE MATCHED MINERAL IN FILE "MATMIN"

- Read in particle width

- Read data on each mineral from file "DIFDAT"
- Included in mineral data are up to eight elemental possibilities with upper and lower bounds on atomic concentration

- Extract from mineral data up to four mandatory elements from first four elemental possibilities listed
- From the spectral peak areas, the ratio of atomic concentrations to silicon are calculated, and then using the average number of silicon atoms in mineral, the atomic concentrations are calculated
- Match up the spectrum-elements with mandatory mineral-elements
- Atomic concentrations must be within range of 80% of lower mineral atomic concentrations and 120% of upper mineral atomic concentrations (sodium has 50% - 150% limits)
- All mandatory mineral-elements must be matched with the exception of a mineral-element with zero lower atomic concentration. (Only one matching failure is allowed. This exception allows for mineral end members)

- Extract from mineral data up to four optional elements from last four elemental possibilities listed
- Compare unused spectrum-elements with optional mineral-elements
- To be matched the atomic concentration must be less than 120% of upper mineral atomic concentration (150% for sodium)
- If concentration is between 120% of upper atomic concentration and upper limit plus 12.5% of average number of silicon atoms in mineral, then this is a conditional failure of which a maximum of 2 are allowed
- If concentration is greater than upper limit plus 12.5% of average number of silicon atoms in mineral, then a match between mineral and spectrum is rejected outright. (If the spectrum-element is not included in the optional mineral-elements, then the upper limit is defined as zero)
- A sum total of three unused spectrum-elements and optional mineral-elements are allowed

WRITE THE MATCHED MINERAL IN FILE "MATMIN"

Figure 17. Descriptive flowchart of computer program to match X-ray spectrum with stored mineral data

```
                                                        DATE: 08-JUN-82

                     PARTICLE IDENTIFICATION


PARTICLE: UNKNOWN SAMPLE X

   WIDTH OF PARTICLE:      0.700  micrometers

   X-RAY SPECTRUM:    ELEMENT     PEAK AREA      ELEMENT     PEAK AREA

                        SI         5325.00          FE        2157.00
                        NA          597.00


    CALCULATED        ELEMENT       RATIO        ELEMENT       RATIO
   ATOMIC RATIOS:
                        SI          1.000           FE          0.425
                        NA          0.430



   MINERALS WITH COMPOSITIONS CONSISTENT WITH X-RAY SPECTRUM

  AEGIRINE                      NA FE SI2 O6
  CROSSITE                      NA2 (MG,FE)3 (FE,AL)2 SI8 O22 (OH)2
  FE-RICHTERITE                 NA CA NA FE5 SI8 O22 (OH)2
  RIEBECKITE                    NA2 FE3 FE2 SI8 O22 (OH)2
```

Figure 18. Computer program output obtained from XMATCH program for input of quantitative EDXA elements from a fiber of riebeckite

mineral is still reported, measurements from an additional zone axis SAED pattern can be entered, together with the tilt angle observed between the positions at which the two patterns were obtained. For the riebeckite example, two zone axis patterns which were obtained by tilting a single fiber about its axis are shown in Figure 19. The angle between the positions at which these patterns were obtained was 21°. Figures 20 and 21 show the solutions obtained from these two patterns. Figure 22 shows the only consistent solutions which remain after permuting the solutions from the two patterns and calculating the inter-zone axis angles. The cell constants for the three minerals are very similar, and discrimination between them on the basis of SAED methods alone would be very difficult.

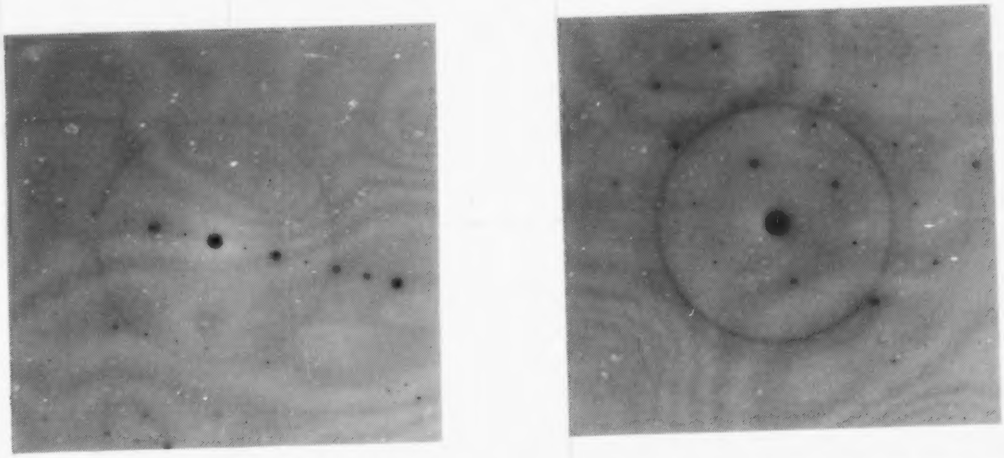
Even using the computer program, the identification procedure is time-consuming; however, it is likely that the SAED data could be transferred directly from the TEM to the computer without photographing the patterns. Thus, an "on-line" particle identification system can be envisaged which incorporates both the EDXA and SAED measurements. For the majority of fibers in a sample, it will not be economic to attempt the complete zone axis identification procedure, and a lower level of identification will have to be accepted in most samples. In general, the complete procedure will be used only to establish the presence of a particular mineral species, and other fibers will then be identified on the basis of morphological, crystallographic, or chemical similarity. Once a particular EDXA spectrum has been associated with a completely identified mineral species in a sample, similar spectra from other particles in the same sample need not be processed by the computer program in order to qualify as quantitative analyses.

7.6 FIBER CLASSIFICATION CATEGORIES

It is not always possible to proceed to a definitive identification of a fiber; this may be due to instrumental limitations, obstruction by sample support grid bars, or the actual nature of the fiber. In many analyses a definitive identification of each fiber may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure must therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system of fiber classification has been devised to permit accurate recording of data.

In this identification protocol the general principle is to establish the most specific fiber classification (target classification) which is to be attempted, and then to record for each fiber the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibers as "identified" can then be established at any time after completion of the analysis.

The classifications as shown in Tables 25 and 26 are directed towards identification of chrysotile and amphibole fibers respectively. In an unknown sample chrysotile can be regarded as confirmed only if a recorded, calibrated SAED pattern from a representative fiber in the CD category



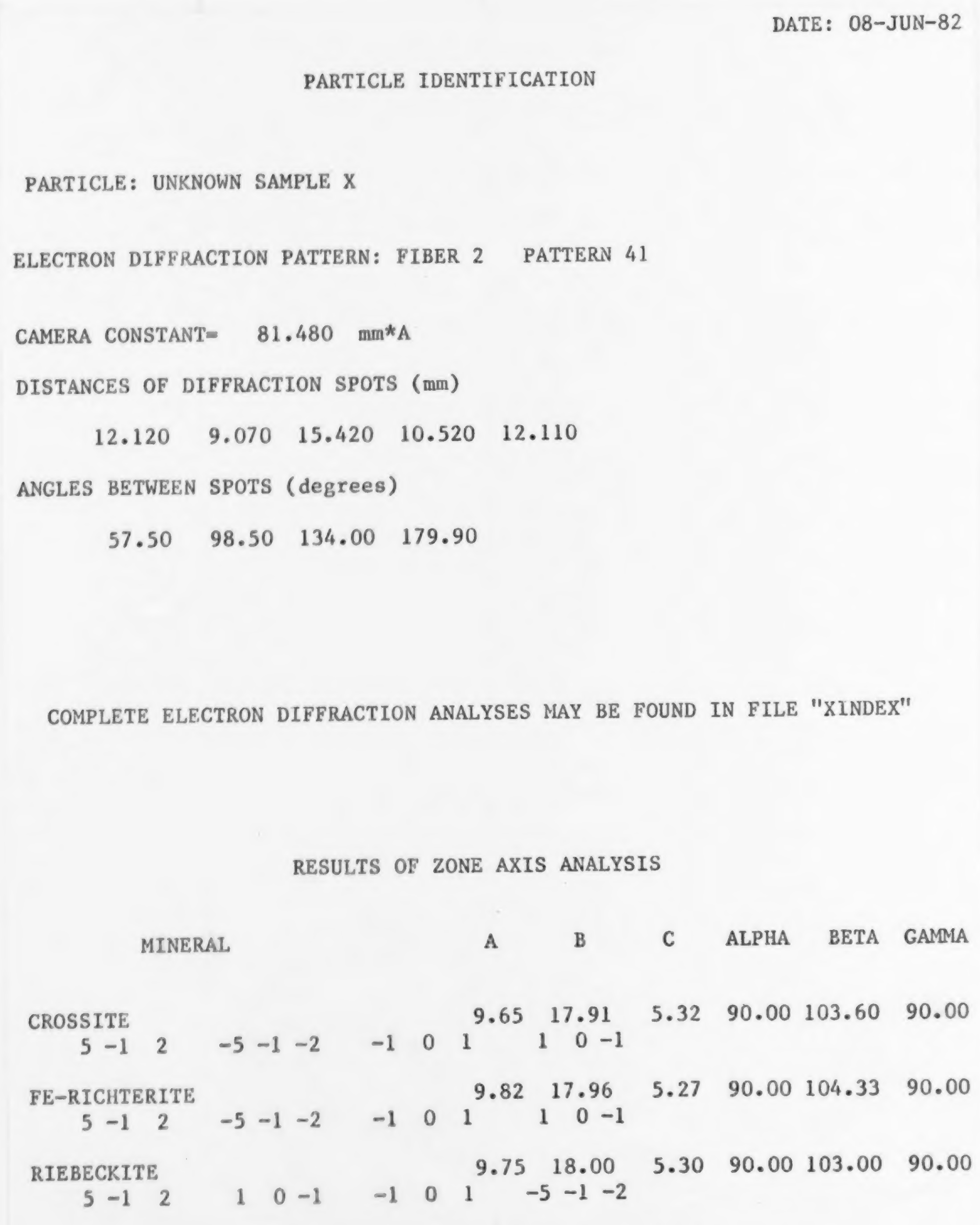
Electron Diffraction Pattern 34

Electron Diffraction Pattern 41

Figure 19. Two zone axis patterns obtained by tilting a single fiber about its axis.

```
                                                          DATE: 08-JUN-82

                         PARTICLE IDENTIFICATION


 PARTICLE: UNKNOWN SAMPLE X

ELECTRON DIFFRACTION PATTERN: FIBER 2   PATTERN 34

CAMERA CONSTANT=   83.030  mm*A

DISTANCES OF DIFFRACTION SPOTS (mm)

         4.580  15.520  30.700  15.520   4.580

ANGLES BETWEEN SPOTS (degrees)

         80.70   89.80   97.50  180.00



   COMPLETE ELECTRON DIFFRACTION ANALYSES MAY BE FOUND IN FILE "XINDEX"



                        RESULTS OF ZONE AXIS ANALYSIS

          MINERAL                           A       B       C     ALPHA   BETA   GAMMA

 CROSSITE                                  9.65   17.91    5.32   90.00  103.60  90.00
     -2  0 -1     2  0  1

 FE-RICHTERITE                             9.82   17.96    5.27   90.00  104.33  90.00
      2  0  1    -2  0 -1

 RIEBECKITE                                9.75   18.00    5.30   90.00  103.00  90.00
      2  0  1    -2  0 -1
```

Figure 20. Solutions obtained from analysis of zone axis pattern 34 shown in Figure 19.



```
                                                              DATE: 08-JUN-82

                           PARTICLE IDENTIFICATION


 PARTICLE: UNKNOWN SAMPLE X

ELECTRON DIFFRACTION PATTERN: FIBER 2   PATTERN 41

CAMERA CONSTANT=   81.480  mm*A

DISTANCES OF DIFFRACTION SPOTS (mm)

     12.120   9.070  15.420  10.520  12.110

ANGLES BETWEEN SPOTS (degrees)

      57.50   98.50  134.00  179.90



  COMPLETE ELECTRON DIFFRACTION ANALYSES MAY BE FOUND IN FILE "X1NDEX"



                       RESULTS OF ZONE AXIS ANALYSIS

        MINERAL                             A       B       C    ALPHA    BETA  GAMMA


 CROSSITE                                 9.65   17.91    5.32   90.00  103.60  90.00
     5 -1  2     -5 -1 -2     -1  0  1      1  0 -1

 FE-RICHTERITE                            9.82   17.96    5.27   90.00  104.33  90.00
     5 -1  2     -5 -1 -2     -1  0  1      1  0 -1

 RIEBECKITE                               9.75   18.00    5.30   90.00  103.00  90.00
     5 -1  2      1  0 -1     -1  0  1     -5 -1 -2
```

Figure 21. Solutions obtained from analysis of zone axis pattern 41 shown in Figure 19.

```
                                                      DATE: 08-JUN-82

                       PARTICLE IDENTIFICATION


  PARTICLE: UNKNOWN SAMPLE X


    ELECTRON DIFFRACTION PATTERNS:

        #1:  FIBER 2   PATTERN 34
        #2:  FIBER 2   PATTERN 41

    MEASURED INTER-ZONE AXIS ANGLE=  21.00  +/-   6.00   degrees



 COMPLETE INTER-ZONE AXIS ANGLE ANALYSIS MAY BE FOUND IN FILE "PHIDAT"



              RESULTS OF INTER-ZONE AXIS ANGLE ANALYSIS

        MINERAL                     ZONE AXIS OF #1  ZONE AXIS OF #2  ANGLE


 CROSSITE                             -2  0 -1         -5 -1 -2       21.14
 CROSSITE                              2  0  1          5 -1  2       21.14
 FE-RICHTERITE                         2  0  1          5 -1  2       20.90
 FE-RICHTERITE                        -2  0 -1         -5 -1 -2       20.90
 RIEBECKITE                            2  0  1          5 -1  2       20.99
 RIEBECKITE                           -2  0 -1         -5 -1 -2       20.99
```

Figure 22. Only solutions remaining after permuting solutions from both patterns 34 and 41 and calculating inter-zone axis angles.

TABLE 25. CLASSIFICATION OF FIBERS WITH TUBULAR MORPHOLOGY

TM	-	Tubular morphology not sufficiently characteristic for classification as chrysotile
CM	-	Characteristic chrysotile morphology
CD	-	Chrysotile SAED pattern
CQ	-	Chrysotile composition by quantitative EDXA
CMQ	-	Chrysotile morphology and composition by quantitative EDXA
CDQ	-	Chrysotile SAED pattern and composition by quantitative EDXA
NAM	-	Non-asbestos mineral

TABLE 26. CLASSIFICATION OF FIBERS WITHOUT TUBULAR MORPHOLOGY

UF	-	Unidentified fiber
AD	-	Amphibole by random orientation SAED (shows layer pattern of 0.53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random orientation SAED and qualitative EDXA
AQ	-	Amphibole by quantitative EDXA
AZ	-	Amphibole by one zone axis SAED
ADQ	-	Amphibole by random orientation SAED and quantitative EDXA
AZQ	-	Amphibole by one zone axis SAED pattern and quantitative EDXA
AZZ	-	Amphibole by two zone axis SAED patterns with consistent inter-axial angle
AZZQ	-	Amphibole by two zone axis SAED patterns, consistent inter-axial angle and quantitative EDXA
NAM	-	Non-asbestos mineral

is obtained. Amphibole can be regarded as confirmed only by obtaining recorded data which yield *exclusively amphibole solutions* for fibers classified in the AZQ, AZZ or AZZQ categories.

7.6.1 Classification of Fibers with Tubular Morphology, Suspected to be Chrysotile

Many fibers are encountered which have tubular morphology similar to that of chrysotile, but which defy further attempts at characterization by either SAED or EDXA. They may be non-crystalline, in which case SAED techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fiber may be of organic origin, but not sufficiently definitive that it can be disregarded. Classification attempts for individual fibers of the same mineral will meet with various degrees of success. Figure 23 shows the classification procedure used for fibers which display any tubular morphology. The chart is self-explanatory, and essentially every fiber is eventually rejected as a non-asbestos mineral (NAM), or classified in some way which could still contribute to the chrysotile fiber count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, the initial classification is TM. Regardless of the doubtful morphology, the fiber is still examined by SAED and EDXA methods according to Figure 23. It may be possible to classify the fiber as having chrysotile morphology (CM) if it possesses the following morphological characteristics.

a) The individual fibrils should have high aspect ratios exceeding 10:1 and be about 40 nm in diameter.

b) The electron scattering power of the fiber at 60 to 100 kV accelerating potential should be sufficiently low for internal structure to be visible.

c) There should be some evidence of internal structure suggesting a tubular appearance similar to that shown in Figure 24A, which may degrade in the electron beam to the appearance shown in Figure 24B.

Every fiber having these morphological characteristics should be examined by the SAED technique, and only those which give diffraction patterns with the precise characteristics of Figure 25 should be classified as chrysotile by SAED (CD). The relevant features in this pattern for identification of chrysotile are indicated in Figure 25. The (002) reflections should be examined to determine that they correspond approximately to a spacing of 0.73 nm, and the layer line repeat distance should correspond to 0.53 nm. There should also be "streaking" of the (110) and (130) reflections. Using millimeter calibrations on the microscope viewing screen,

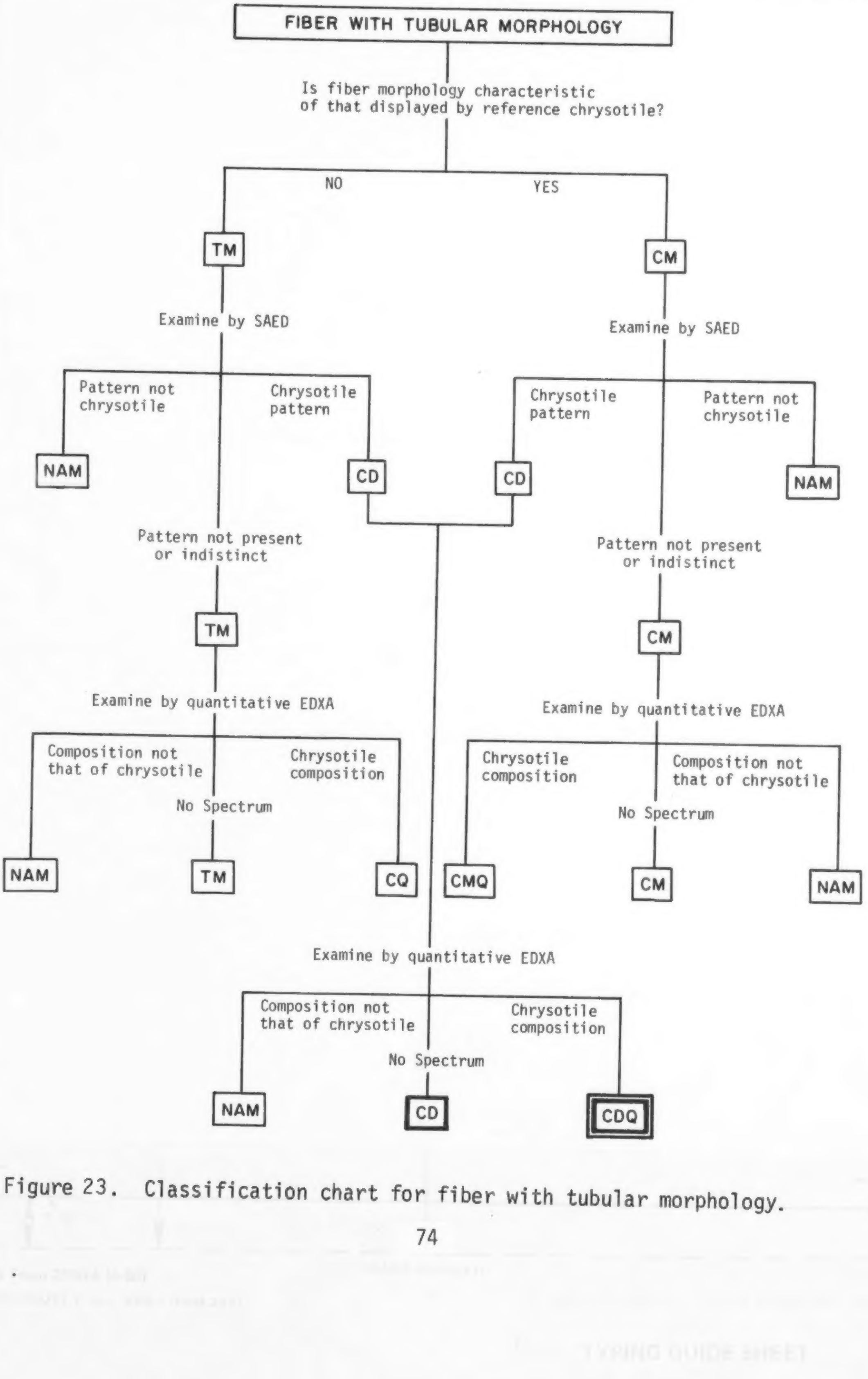

FIBER WITH TUBULAR MORPHOLOGY
Is fiber morphology characteristic of that displayed by reference chrysotile?
NO
YES
TM
CM
Examine by SAED
Examine by SAED
Pattern not chrysotile
Chrysotile pattern
Chrysotile pattern
Pattern not chrysotile
NAM
CD
CD
NAM
Pattern not present or indistinct
Pattern not present or indistinct
TM
CM
Examine by quantitative EDXA
Examine by quantitative EDXA
Composition not that of chrysotile
Chrysotile composition
No Spectrum
Chrysotile composition
Composition not that of chrysotile
No Spectrum
NAM
TM
CQ
CMQ
CM
NAM
Examine by quantitative EDXA
Composition not that of chrysotile
Chrysotile composition
No Spectrum
NAM
CD
CDQ

Figure 23. Classification chart for fiber with tubular morphology.

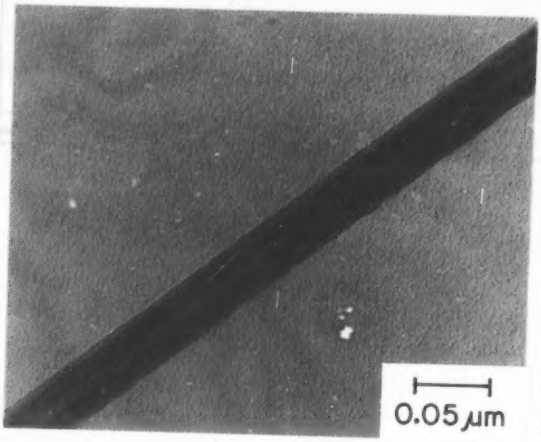


Figure 24A. TEM micrograph of chrysotile fibril, showing morphology.

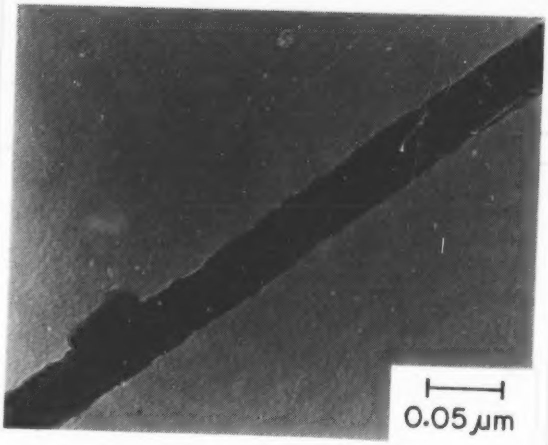


Figure 24B. TEM micrograph of UICC Canadian chrysotile fiber after thermal degradation by electron beam irradiation.

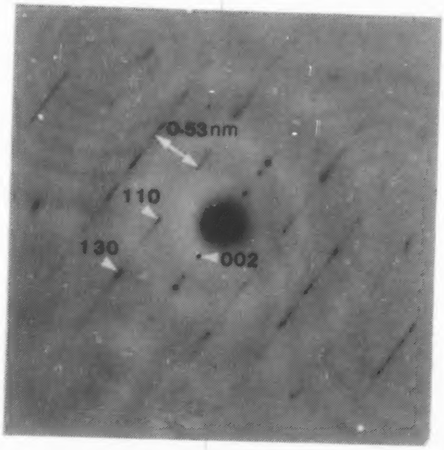


Figure 25. SAED pattern of chrysotile fiber with diagnostic features labelled. Necessary criteria are the presence of 0.73 nm spacing for the 002 reflections, 0.53 nm spacing for the layer line repeat and characteristic streaking of the 110 and 130 reflections.

these observations can readily be made at the instrument. A TEM micrograph of at least one representative fiber should be recorded, and its SAED pattern should also be recorded on a separate film or plate. This plate must carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the *only* documentary proof that the particular fiber is chrysotile and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite.

The proportion of fibers which can be successfully identified as chrysotile by SAED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibers that fail to yield an identifiable SAED pattern therefore remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile, there are only two elements which are relevant. For fiber classification, the EDXA analysis should be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, *with their areas in the appropriate ratio*, and with only minor peaks from other elements, the fiber should be classified as chrysotile by quantitative EDXA, in the categories CQ, CMQ or CDQ, as appropriate.

For chrysotile analyses there are essentially three possible levels of analysis which are shown below:

1. morphological and SAED discrimination only (target classification CD);
2. in addition, EDXA of only those fibers which remained unclassified by SAED (target classification CD);
3. EDXA in addition to SAED of all fibers (target classification CDQ).

7.6.2 Classification of Fibers Without Tubular Morphology, Suspected to be Amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 3 to 1 or greater, and having parallel or stepped sides, should be considered as a suspected amphibole fiber. Further examination of the fiber by SAED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fiber and on a number of instrumental limitations. It will not be possible to identify every fiber completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved *only* by quantitative interpretation of zone axis SAED patterns, a very time-consuming procedure. For routine samples from unknown sources, zone axis SAED work should be performed on at least one fiber typical of each compositional class reported. When a higher degree of certainty is required, it may be necessary to identify more fibers by the zone axis technique. When analyzing samples from well-characterized sources, the cost of identification by zone axis methods may not be justified.

The 0.53 nm layer spacing of the random orientation SAED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibers leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, as shown in Figure 26, if also associated with a high fiber aspect ratio, is a characteristic of some types of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fiber is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fiber may be of some other mineral species.

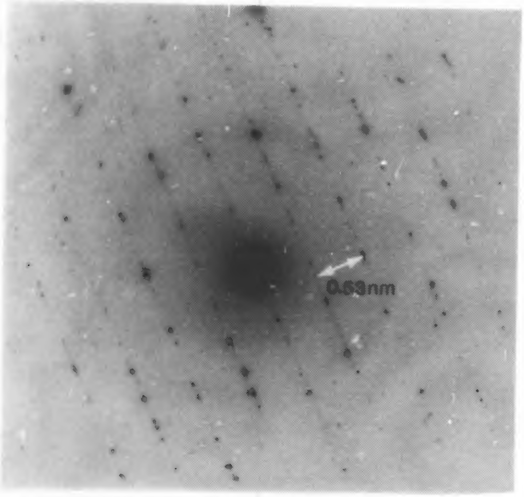


Figure 26. Amphibole SAED pattern (crocidolite) obtained from a fiber without precise orientation onto a zone axis.

As for chrysotile, the procedure is first to define the target level of classification to be attempted, and then to record the degree of success achieved for each fiber. Figure 27 shows the fiber classification chart for suspected amphibole fibers. This chart shows all the classification paths possible in the analysis of a suspected amphibole fiber, when examined systematically by SAED and EDXA. Initially, two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation SAED pattern is made first. The normal procedure for analysis of a sample of unknown origin is to examine the fiber by random orientation SAED, qualitative EDXA, quantitative EDXA, and

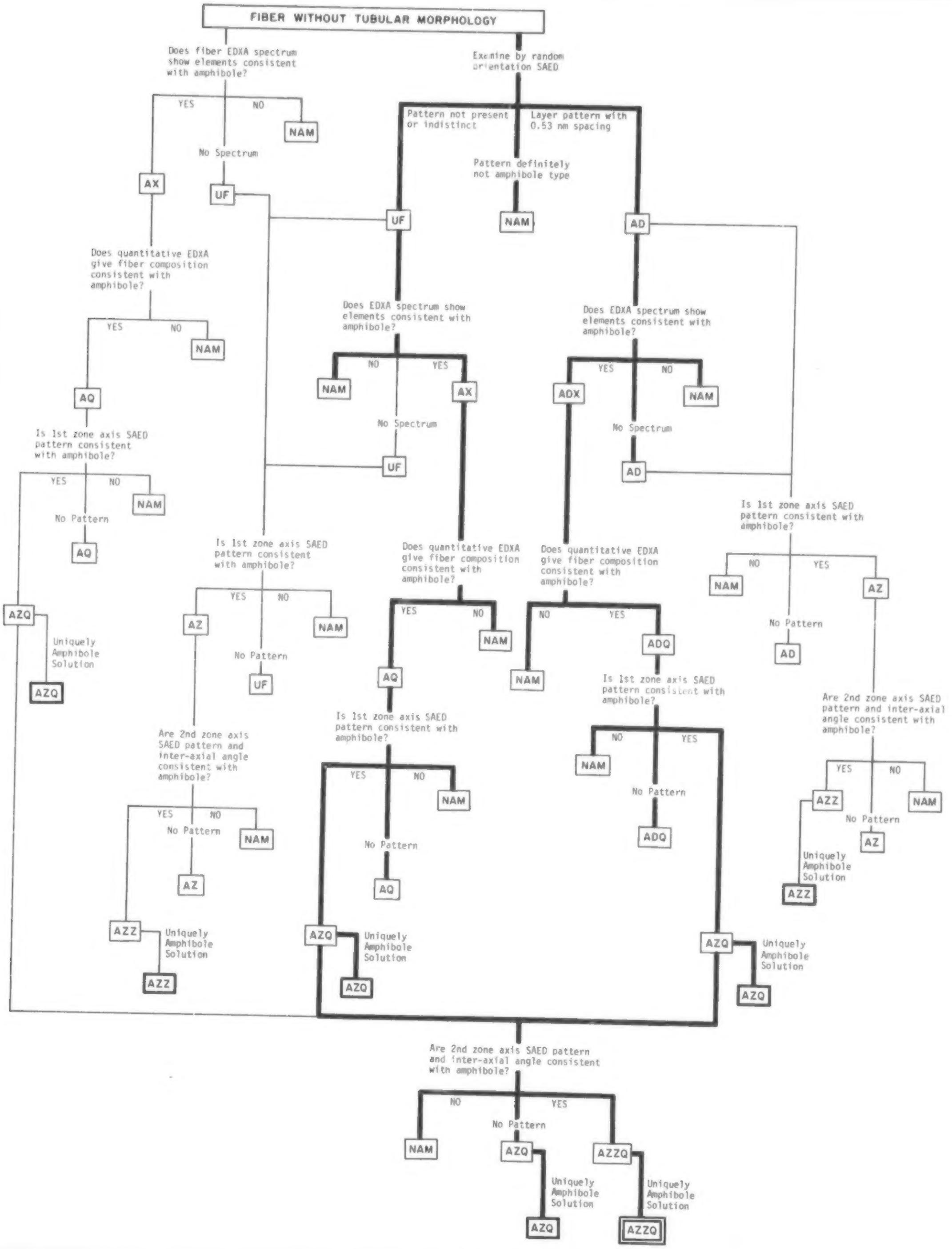

FIBER WITHOUT TUBULAR MORPHOLOGY
Does fiber EDXA spectrum show elements consistent with amphibole?
YES
NO
NAM
No Spectrum
AX
UF
Examine by random orientation SAED
Pattern not present or indistinct
Layer pattern with 0.53 nm spacing
Pattern definitely not amphibole type
UF
NAM
AD
Does quantitative EDXA give fiber composition consistent with amphibole?
YES
NO
NAM
AQ
Is 1st zone axis SAED pattern consistent with amphibole?
YES
NO
NAM
No Pattern
AQ
AZQ
Uniquely Amphibole Solution
AZQ
Does EDXA spectrum show elements consistent with amphibole?
NO
YES
NAM
AX
No Spectrum
UF
Is 1st zone axis SAED pattern consistent with amphibole?
YES
NO
AZ
NAM
No Pattern
UF
Are 2nd zone axis SAED pattern and inter-axial angle consistent with amphibole?
YES
NO
No Pattern
NAM
AZ
AZZ
Uniquely Amphibole Solution
AZZ
Does quantitative EDXA give fiber composition consistent with amphibole?
YES
NO
NAM
AQ
Is 1st zone axis SAED pattern consistent with amphibole?
YES
NO
NAM
No Pattern
AQ
AZQ
Uniquely Amphibole Solution
AZQ
Does EDXA spectrum show elements consistent with amphibole?
YES
NO
ADX
NAM
No Spectrum
AD
Does quantitative EDXA give fiber composition consistent with amphibole?
NO
YES
ADQ
NAM
Is 1st zone axis SAED pattern consistent with amphibole?
NO
YES
NAM
No Pattern
ADQ
AZQ
Uniquely Amphibole Solution
AZQ
Is 1st zone axis SAED pattern consistent with amphibole?
NO
YES
NAM
AZ
No Pattern
AD
Are 2nd zone axis SAED pattern and inter-axial angle consistent with amphibole?
YES
NO
AZZ
NAM
No Pattern
AZ
Uniquely Amphibole Solution
AZZ
Are 2nd zone axis SAED pattern and inter-axial angle consistent with amphibole?
NO
YES
No Pattern
NAM
AZQ
AZZQ
Uniquely Amphibole Solution
Uniquely Amphibole Solution
AZQ
AZZQ

Figure 27. Classification chart for fiber without tubular morphology.

zone axis SAED, in this sequence. The final fiber classification assigned will be defined either by successful analysis at the target level or by the instrumental limitations. The maximum classification achieved for each fiber should be recorded. The various classification categories can then be combined later in any desired way for calculation of the fiber concentration, and a complete record of the results from each fiber is maintained for re-assessment of the data, if necessary.

Depending on the particular analytical requirements, four levels of analysis can be defined in this analytical procedure, and these levels are shown in Table 27.

In the routine unknown sample, a level 3 analysis is required if the presence of amphibole is to be confirmed. For this level of analysis, attempts are made to raise the classification of every fiber to the ADQ category. In addition, at least one fiber from each type of suspected amphibole found must be examined by zone axis SAED methods to confirm the identification.

TABLE 27. LEVELS OF ANALYSIS FOR AMPHIBOLE

Level of Analysis	Application	Target Classification for all Fibers	Required Classification for Confirmation of Amphibole in a Proportion of the Fibers
1	Routine monitoring of known and well-characterized sources for one mineral fiber type.	ADX	Not Applicable
2	Routine monitoring of known and well-characterized sources where discrimination between two or more amphibole fiber types is required.	ADQ	Not Applicable
3	Routine samples from uncharacterized sources in which presence or absence of amphibole is to be confirmed.	ADQ	AZZ, AZQ or AZZQ - Solutions must include only amphiboles.
4	Samples where precise identification of all amphibole fibers is an important issue.	AZQ	AZZQ - Solutions must include only amphiboles.

7.6.3 Reporting of Fiber Classifications

Before the fiber count data can be processed to give concentration values, a decision must be made as to which fiber classifications are to be considered adequate as identification of the fiber species in question. This decision will depend on how much is known about the particular source from which the sample was collected.

If the sample was collected from a completely uncharacterized source, it is recommended that the classified fibers be grouped as below.

a)	Confirmed Chrysotile:	CDQ + CD
b)	Chrysotile Best Estimate:	CDQ + CD + CMQ + CQ
c)	Suspected Chrysotile:	CM
d)	Confirmed Amphibole:	AZZQ + AZQ + AZZ (Solutions must include only amphiboles)
e)	Amphibole Best Estimate:	AZZQ + AZQ + AZZ + AZ + ADQ + AQ
f)	Suspected Amphibole:	ADX + AX + AD

The *Best Estimate* should be reported only if some fibers are also reported in the confirmed category, otherwise all fiber classifications can only be considered as *suspected* amphibole or chrysotile.

The classification procedure defined has been found to be practical, and accommodates both the inherent instrumental limitations and the limited mineralogical training of most TEM operators. Using this procedure, the decision as to what combination of measurements constitutes an adequate identification of a fiber can be made after the fiber count has been made, and upgrading of the fiber classifications can be made by subsequent review or additional measurements, if necessary. Moreover, TEM operators have appreciated the transfer of the onus for fiber identification to the user of the data, and interpretation of data from different operators or from different samples is considerably easier, more complete, and more reliable.

SECTION 8

DETERMINATION OF FIBROSITY

The data available on adverse health effects due to exposure to asbestos fibers are largely confined to considerations of exposure to those minerals which are exploited commercially. Consequently, regulations regarding maximum permissible levels in workplace atmospheres usually refer to "asbestos" fibers, where "asbestos" is defined as one of the commercial varieties. When fiber concentrations are measured in industries where the commercial varieties are not in use, or in the general environment, the question arises as to whether a particular fiber is "asbestos". The amphibole asbestoses all have non-fibrous counterparts which display very prominent cleavage parallel to the c-axis, and crushing of these minerals yields a large number of elongated cleavage fragments. These fragments are chemically identical to asbestos fibers which originate from the fibrous version of the mineral. Thus, EDXA measurements are incapable of discriminating between them. The short range crystallography of the cleavage fragment is also identical to that of the fibrous version, although in the latter there is often substantial twinning parallel to the c-axis which occasionally may allow some discrimination in the TEM between the two types.[5] However, for routine sample analysis there appears to be no simple way to assign a single fiber of these materials as either "asbestos" or "cleavage fragment".[46]

It has been suggested that this ambiguity of identification, and the possible confusion of minerals such as cummingtonite and minnesotaite, could be resolved by increasing the aspect ratio for definition of a fiber.[47] The value of 10:1 has been discussed, but the suggestion has received little support. The aspect ratio-cumulative number distribution for an aqueous dispersion of UICC amosite, shown in Figure 28, indicates that use of the 10:1 aspect ratio definition would involve rejection of about 80% of the fibers counted under the current 3:1 aspect ratio definition. While rejection of such a large proportion of the particles is considered unacceptable by most administrations, it is thought necessary to be able to discriminate asbestos fibers from cleavage fragments.

It is clear that discrimination between hand samples of the fibrous and non-fibrous minerals presents no difficulty. It is only when the maximum fiber length is small that the ambiguity appears. At low magnifications on the optical microscope, it is still relatively easy to determine whether amphibole particles are fibrous or whether they are cleavage fragments. However, when the size distribution of the fibers is such that the TEM is required for the observation, there is usually a large proportion of low aspect ratio fibers, regardless of whether they originate from the fibrous or the non-fibrous mineral.

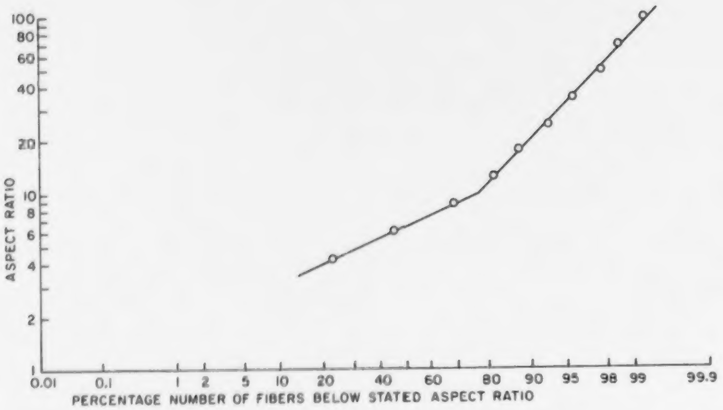


Figure 28. Aspect ratio distribution for an aqueous dispersion of UICC amosite.

The concept of an "index of fibrosity" has been discussed by Wylie[35] and it was decided to investigate this approach further. The description of a material as "fibrous" requires that some proportion of the fibers have high aspect ratios, and conversely, a high median aspect ratio for the population of fibers indicates a high degree of fibrosity. If there is a large proportion of low aspect ratio fibers, as is the case for the amosite distribution in Figure 28, the median aspect ratio will be relatively low, but the range of aspect ratio above the median may be very large and the material will still be considered as fibrous. Accordingly, the description of fibrosity will involve the median aspect ratio and the standard deviation of the aspect ratio distribution above the median. To evaluate this concept, aqueous dispersions of a number of fibrous and non-fibrous minerals were prepared. The minerals were first ground using a mortar and pestle, after which they were dispersed in double-distilled water. TEM samples were then prepared from the dispersions by the carbon-coated Nuclepore procedure. Fiber aspect ratio distributions were obtained by measuring the lengths and widths of about 250 fibers (aspect ratios exceeding 3:1) from each sample. The aspect ratio versus cumulative number distributions were plotted in the same way as for Figure 28. It was observed that although the aspect ratio distributions for most non-fibrous minerals were approximately logarithmic-normal, those of the fibrous minerals frequently deviated from this approximation as illustrated in Figure 28.

To describe the fibrosity of a population of particles, the properties of the empirical function:

$$F = A^{g}$$

were investigated, where A is the median aspect ratio, and g is the geometric standard deviation in the region above the median. The value of g was obtained by extrapolation of the region of the graph between the 84.13% and 97.73% points, corresponding to the region between one and two standard deviations above the median.

Table 28 shows values of the median aspect ratio, geometric standard deviation between the 84.13% and 97.73% points, and the calculated fibrosity index F for a range of non-fibrous and fibrous minerals. It can be seen that the non-fibrous minerals have values below about 50 and the values for the known asbestos varieties are very high. This fibrosity index can be derived from the measurement of only 50 fibers in some cases, but about 150 - 350 fibers were measured to obtain the values in Table 28. It is important to recognize that this fibrosity index is simply an empirical number which describes the aspect ratio distribution *in the particular sample*, and that the value can be lowered by selective removal of the high aspect ratio fibers. Low values are also obtained if the number of fibers measured is too low to define adequately the high aspect ratio side of the distribution. With these reservations, the fibrosity index derived from TEM fiber counts can be used in most cases to discriminate between particle populations of fibrous and non-fibrous mineral species.

Anthophyllite seems to be an exception to this rule, and material from three sources was examined. The aspect ratio distribution of UICC anthophyllite yielded the lowest fibrosity index encountered, and inspection of the data showed that there were no large aspect ratio fibers on the TEM samples. Anthophyllite from two other sources was examined and although the values of the fibrosity indices correlated with the appearances of the bulk mineral samples, the aspect ratio distributions in the TEM size range were not similar to those from the other commercially-exploited asbestos varieties.

TABLE 28. INDICES OF FIBROSITY FOR SELECTED MINERALS

Mineral Fiber	Number of Fibers Counted	Median Aspect Ratio	Geometric Standard Deviation above 84.13% Point	Fibrosity Index F
Grunerite	330	5.1	1.76	17.7
Cummingtonite	370	5.1	1.82	19.5
Tremolite	349	5.6	1.75	20.4
Hornblende	329	5.75	1.88	26.7
Actinolite	308	5.70	1.87	25.8
Riebeckite	308	4.9	2.38	43.9
Anthophyllite (UICC)	315	4.95	1.69	14.9
Anthophyllite (Quebec)	391	6.35	1.91	33.9
Anthophyllite (Salt Mt.)	303	5.34	2.68	89.4
UICC Amosite	140	6.3	3.48	604.9
Union Carbide Chrysotile	141	21.0	2.29	1066.3
UICC Crocidolite	199	7.8	2.81	321.2
Beaver Bay Water (Amphibole)	181	6.4	3.33	483.7
Taconite Tailings	673	6.7	2.89	241.8
Sherbrooke Drinking Water (Chrysotile)	197	13.0	2.23	304.9
Tremolite in Vermiculite Sample	330	12.0	2.45	441.0

SECTION 9

STATISTICS OF FIBER COUNTING

9.1 TEST FOR UNIFORMITY OF FIBER DEPOSIT ON ELECTRON MICROSCOPE GRIDS

A check should be made using the chi-square test, to determine whether the fibers found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number of fibers found in k grid openings is n, and the areas of the k individual grid openings are designated A_1 to A_k, then the total area examined is:

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, p_i, is given by A_i/A. If the fibers are randomly and uniformly dispersed over the k grid openings counted, the expected number of fibers falling in one grid opening with area A_i is np_i. If the observed number found on that grid opening is n_i, then:

$$\chi^2 = \sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

This value is compared with significance points of the χ^2 distribution, having (k - 1) degrees of freedom. Significance levels lower than 0.1% are caused for the sample analysis to be rejected, since this corresponds to a very inhomogeneous deposit. If this occurs, a new filter should be prepared, paying more attention to both uniform dispersal of the suspension and the filtration procedure.

9.2 CALCULATION OF THE MEAN AND CONFIDENCE INTERVAL OF THE FIBER CONCENTRATION

In a fiber count a maximum of 20 grid openings is usually sampled from a population of grid openings, and it is required to determine the mean grid opening fiber count for the population on the basis of this sampling. The interval about the sample mean, which, with 95% confidence, contains the population mean, is also required. In previous work[7,23] Gaussian statistics were used for this calculation because it had been noted that the fiber counts were frequently not Poissonian. However, at low fiber counts, this assumption leads to lower 95% confidence limits which are negative. The distribution for low fiber counts is certainly skewed if not accurately Poissonian. Assumption of the Poisson distribution, when the distribution is demonstrably not Poissonian, is not correct either. Because some interpretation of low fiber counts is required, the fiber distribution statistics of a large number of samples were reviewed, and a logical procedure was developed.

The distribution of fibers on the grid openings should theoretically approximate to a Poisson distribution. Because of fiber aggregation, filter defects and size-dependent identification effects, the actual data often does not conform to the Poisson distribution, particularly at high fiber counts. Simple assumption of a Poisson distribution may therefore lead to confidence intervals narrower than are justified by the data.[48] Moreover, if a Poisson distribution is assumed, the variance is fixed in relation to the total number of fibers counted. Thus a particular fiber count conducted on one grid opening is considered to have the same confidence interval as that for the same number of fibers found on many grid openings. However, the area of sample actually counted is very small in relation to the total area of the filter, and for this reason fibers must be counted on a minimum of 4 grid openings taken from different areas of the filter in order to ensure representative evaluation of the deposit.

At high fiber counts, where there are adequate numbers of fibers per grid opening to allow a sample estimate of the variance to be made, the distribution can be approximated to Gaussian, with independent values for the mean and variance. Where the sample estimate of variance exceeds that implicit in the Poissonian assumption, use of Gaussian statistics with the variance defined by the actual data is the most conservative approach to calculation of confidence intervals.

At low fiber counts it is not possible to obtain a reliable sample estimate of the variance, and the distribution also becomes asymmetric, but not necessarily Poissonian. For 30 fibers and below, the distribution becomes sufficiently asymmetric that the Gaussian fit is no longer a reasonable one, and sample variance estimates are unreliable. Accordingly, for fiber counts below 31 fibers, the assumption of a Poisson distribution must be made for calculation of the confidence intervals. Table 29 shows the 95% confidence limits for selected numbers of fibers, assuming the Poisson distribution. For total fiber counts less than 4, the lower 95% confidence value corresponds to less than one fiber, and in addition, the upper 95% confidence value corresponding to a fiber count of zero is 3.69 fibers. Therefore, it is not meaningful to quote lower confidence interval points for fiber counts of less than 4, and the result should be specified as "less than" the corresponding Poisson upper 95% confidence value. For fiber counts higher than 30, the sample estimate of variance can be calculated, and the larger of the two confidence intervals may be selected. For calculation of Poisson 95% confidence intervals, Table 40 of the reference by E.S. Pearson and H.O. Hartley[49] should be used, with an extension to an expectation of 100.

TABLE 29. 95% CONFIDENCE LIMITS FOR THE POISSON DISTRIBUTION

Observed Number of Fibers	Lower 95% Confidence Limit	Upper 95% Confidence Limit
0	0.00	3.69
1	0.025	5.57
2	0.24	7.22
3	0.62	8.77
4	1.09	10.24
5	1.62	11.67
10	4.80	18.39
20	12.22	30.89
30	20.24	42.83
40	28.58	54.47
50	37.11	65.92
100	81.4	121.6
200	174	230

For more than 100 fibers, the Poisson distribution can be accurately approximated by a Gaussian distribution, still using the Poisson variance estimate. For counts of more than 30 fibers the 95% confidence interval, based on a sample estimate of variance, is calculated using the Student's "t" distribution. For the two-sided Student's "t" calculation, k values of grid opening fiber count are compared with the expected values for the areas of the grid openings concerned.

In summary, fiber counting data should be reported as follows.

No fibers detected

The value should be reported as less than 369% of the concentration equivalent to one fiber.

1 to 4 fibers

When 1 to 4 fibers are counted, the result should be reported as less than the corresponding upper 95% confidence limit (Poisson).

5 to 30 fibers

Mean and 95% confidence intervals should be reported on the basis of the Poisson assumption.

More than 30 fibers

When more than 30 fibers are counted, both the Gaussian 95% confidence interval and the Poisson 95% confidence interval can be calculated. The larger of these two intervals should be selected for data reporting. When the Gaussian 95% confidence interval is selected for data reporting, the Poisson interval should also be noted.

Fiber counts performed on less than 4 grid openings yield very wide 95% confidence intervals when using Gaussian statistics. This is because the value of Student's "t" is very large for 1 and 2 degrees of freedom. Accordingly, fiber counts should not be made on less than 4 grid openings.

The sample estimate of variance S^2 is first calculated:

$$S^2 = \frac{\sum_{i=1}^{i=k} (n_i - np_i)^2}{(k-1)}$$

where:

n_i = number of fibers on the i'th grid opening

n = total number of fibers found in k grid openings

p_i = fraction of the total area examined represented by the i'th grid opening

k = number of grid openings

For the 95% confidence interval, the value of $t_{0.975}$ is obtained from tables for (k - 1) degrees of freedom. If the mean value of fiber count is calculated to be $\bar{n}$, the upper and lower values of the 95% confidence interval are given by:

$$n_U = \bar{n} + \frac{ts}{\sqrt{k}}$$

$$n_L = \bar{n} - \frac{ts}{\sqrt{k}}$$

where:

n_U = upper 95% confidence limit

n_L = lower 95% confidence limit

$\bar{n}$ = mean number of fibers per grid opening

s = standard deviation (square root of sample estimate of variance)

k = number of grid openings

SECTION 10

DEVELOPMENT OF A REFERENCE METHOD FOR DETERMINATION OF ASBESTOS FIBER CONCENTRATIONS IN WATER

A reference method for determination of asbestos fiber concentrations in water was written, which incorporates many of the results of the research program. Some of the changes or additions made to the EPA Interim Method[2] were major ones, although the fundamental carbon-coated Nuclepore specimen preparation method remained the same.

10.1 USE OF OZONE-ULTRAVIOLET LIGHT TREATMENT FOR OXIDATION OF INTERFERING ORGANIC MATERIALS

Ozone-UV treatment was specified for *all samples*. This involves a 3 - 4 hour period during which 1% ozone is bubbled through the liquid *in the collection container*, while it is also irradiated with 254 nm UV light. This treatment is necessary for the following reasons.

a) A small amount of high molecular weight organic materials, probably polysaccharides, is normally present in drinking water. Particularly if the container is agitated during transportation, these materials, along with the fibers, are collected on the inside surfaces of the container. This scavenging effect is capable of almost complete removal of suspended asbestos fibers, both chrysotile and amphibole. The process is largely irreversible without chemical treatment to oxidize the organics. The proposed treatment has been shown to restore the scavenged fibers to suspension, and to yield reliable recoveries not otherwise possible.

b) Filtration rates through the Nuclepore filters are always improved due to the removal of interfering organics.

c) Removal of the polysaccharide material virtually eliminates the occurrence of uncountable aggregates of fibers.

d) Use of the ozone-UV method virtually eliminates the requirement for low temperature ashing. Low temperature ashing appears to be unreliable and it also concentrates any contamination associated with the filters.

10.2 USE OF ULTRASONIC TREATMENT

Ultrasonic treatment of water samples in a bath-type unit, both before and after ozone-UV treatment, is specified. Use of mild ultrasonic treatment has been found to be the only way of achieving consistent results from identical samples. No evidence has been found that use of ultrasonic treatment at these low powers for the specified times results in measurable fiber disintegration.

10.3 USE OF THE CONDENSATION WASHER FOR REMOVAL OF RESIDUAL UNDISSOLVED PLASTIC FROM TEM SAMPLES

Recent batches of Nuclepore polycarbonate filters are very difficult to dissolve in chloroform in a Jaffe Washer, even after treatment extending over several days. It is found that after the Jaffe dissolution step, residual undissolved plastic remains on the sample such that during the TEM examination a significant proportion of the fibers may not be seen. After consultation with Nuclepore Corporation, it seems unlikely that this situation is going to improve. Use of methylene chloride, or N-methyl-2-pyrrolidone as alternative solvents was suggested. Methylene chloride was found to evaporate very rapidly and required the Jaffe Washer to be tightly sealed. Although it yielded TEM samples which were more satisfactory, it did not completely remove the plastic. When N-methyl-2-pyrrolidone was used, it was found to leave an oily film on the samples, which could only be removed by treatment with another solvent. Moreover, the rapid initial solvent attack on the plastic caused a significant degree of breakage of the carbon film.

The most effective technique was found to be an initial Jaffe Washer dissolution, followed by a short treatment in a condensation washer. Use of the condensation washer as a single dissolution step is not recommended, since this requires that initially chloroform be pipetted directly onto the TEM sample to avoid curling of the filter in the chloroform vapor. Using the original design of Jaffe Washer, the piece of paper under the samples can easily be moved to the cold finger of a condensation washer. After treatment in the condensation washer for 30 to 60 minutes, the TEM samples are then found to be satisfactory. Use of the condensation washer in this way should not incur fiber losses, since all particles are thought to be held in position by the carbon film. However, additional experiments were conducted to demonstrate that this was the case.

The Nuclepore control filters used originally for the collapsed membrane filter study were used to make measurements of any fiber losses incurred by the additional condensation washing step. The control filter results for chrysotile and crocidolite are shown in Tables A-2 and A-5 of Appendix A. Four filters from each series were analyzed again after preparation, using the condensation washing step. The detailed results are shown in Tables D-1 and D-2 of Appendix D. The fiber loss analyses for chrysotile and crocidolite are shown in Tables 30 and 31 respectively.

TABLE 30. CONDENSATION WASHER STUDY: CHRYSOTILE FIBER LOSS ANALYSIS

Sample Preparation	Number of Filters Analyzed	Fiber Concentration (10^6 fibers/liter)		Fiber Loss in Preparation (%)*	Is the Fiber Loss Detectable at 5% Significance?
		Mean	95% Confidence Interval		
Control Samples Prepared by Jaffe Washer only	10	264.8	236.6 - 293.6	-	-
Samples from Same Series prepared by Successive Jaffe and Condensation Washing	4	258.0	208.1 - 307.9	2.6	No

*Compared to the Carbon-Coated Nuclepore Preparation

TABLE 31. CONDENSATION WASHER STUDY: CROCIDOLITE FIBER LOSS ANALYSIS

Sample Preparation	Number of Filters Analyzed	Fiber Concentration (10^6 fibers/liter)		Fiber Loss in Preparation (%)*	Is the Fiber Loss Detectable at 5% Significance?
		Mean	95% Confidence Interval		
Control Samples Prepared by Jaffe Washer only	10	224	158 - 290	-	-
Samples from Same Series prepared by Successive Jaffe and Condensation Washing	4	243	198 - 288	0	No

*Compared to the Carbon-Coated Nuclepore Preparation

It can be seen that no fiber losses could be detected at the 5% significance level.

Figures 29 and 30 show the deposit from an aqueous dispersion containing chrysotile and other materials. These micrographs show the same area of a TEM sample both before and after condensation washing for a period of 180 minutes. It can be seen that no particle losses or movement have occurred as a result of re-washing of the TEM grids in the condensation washer. In the area of the sample illustrated, the residual plastic was almost completely dissolved by the Jaffe Washer treatment. The remaining plastic was not, in this case, removed by the condensation washer, since the initial observation in the TEM caused cross-linking of the plastic and increased its insolubility.

It was concluded that the condensation washing step did not compromise the samples in any way, and that the operating conditions of the washer were

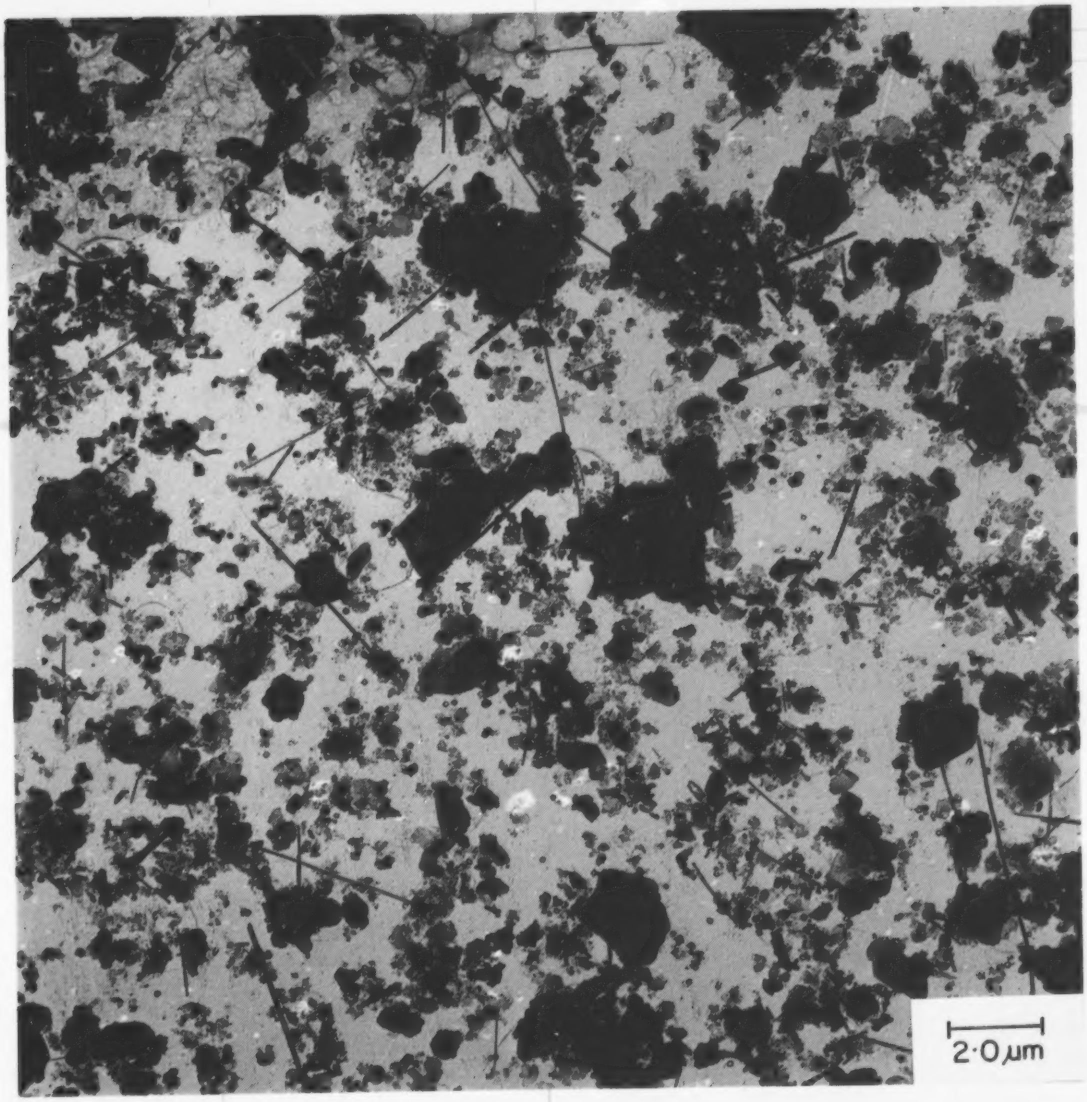


Figure 29. TEM specimen prepared from aqueous suspension of asbestos and other materials. Polycarbonate filter dissolved using chloroform in Jaffe Washer only.

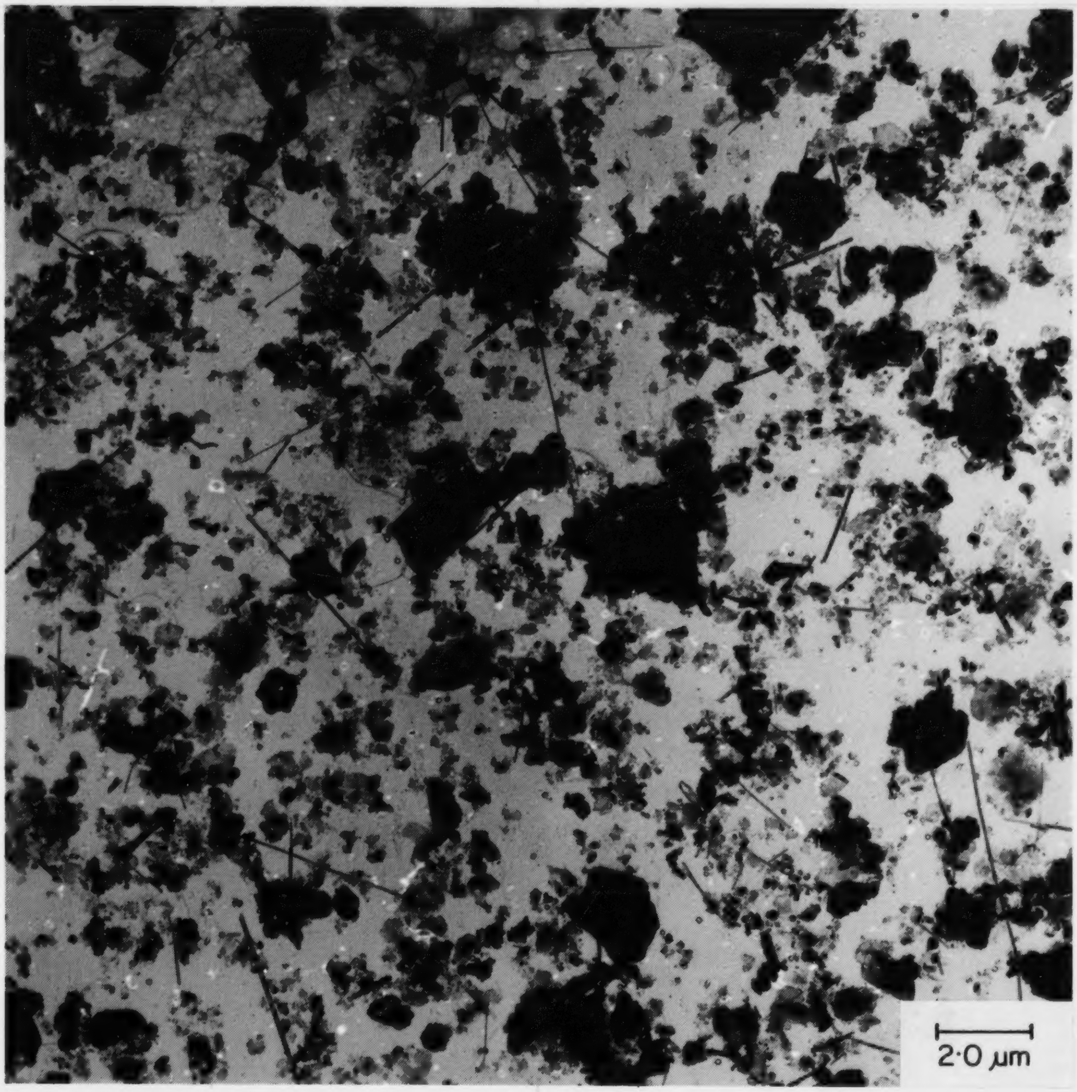


Figure 30. TEM specimen shown in Figure 29, but after further treatment using chloroform in condensation washer for 180 minutes.

not critical. Accordingly, it was incorporated into the analytical method as an optional step in the routine procedure.

10.4 INTRODUCTION OF MINIMUM FIBER LENGTH TO BE REPORTED

A minimum fiber length of 0.5 μm has been specified for the following reasons.

a) Collection of fibers shorter than 0.5 μm by a 0.1 μm nominal pore size Nuclepore filter becomes less efficient, and may be variable depending on both the batch number and the filtration rate.

b) Identification of fibers less than about 0.05 μm in diameter becomes very unreliable.

c) Nuclepore filters contain many artifacts which can be mistaken by inexperienced operators for fibers below 0.5 μm in length.

d) Operators vary in their interpretation when asked to count all fibers, particularly when large numbers of fibers are present.

Counting fibers with lengths less than 0.5 μm is acceptable, however, if they can be identified as asbestos fibers.

10.5 REQUIREMENT FOR QUANTITATIVE INTERPRETATION OF CHRYSOTILE SAED PATTERNS

A gold-ring-calibrated SAED pattern from typical fibers has been specified as part of the report, from which the 0.53 nm layer line spacing and the 0.73 nm (002) spacing can be calculated. These and other relevant features for identification of chrysotile by SAED are also specified. This limited quantitative interpretation is required in order to discriminate chrysotile from halloysite, palygorskite and scrolled vermiculite.

10.6 REQUIREMENT FOR ENERGY DISPERSIVE X-RAY ANALYSIS AND ZONE AXIS SAED FOR AMPHIBOLE IDENTIFICATION

Unequivocal analysis for amphiboles is extremely difficult and can be achieved *only by using the most sophisticated instrumentation*. Therefore, a requirement for an electron beam diameter of less than 100 nm and a suitable energy dispersive spectrometer have been specified. A goniometer stage is also required for this work. At least one indexed and appropriate zone axis SAED pattern and quantitative EDXA measurements from a representative fiber are required as part of the report for confirmation of the presence of amphibole. All other suspected amphibole fibers are classified according to morphological, crystallographic and chemical similarity with the identified fiber.

10.7 INTRODUCTION OF A FIBER CLASSIFICATION SYSTEM

A fiber classification system has been incorporated which requires that the operator specify the identification action taken and its result for each fiber. The system removes the onus of *identification* from the microscope operator, who is instructed to attempt a specified degree of classification by morphology, SAED and EDXA. The classifications are then reviewed by the user of the data, who makes the decision as to what constitutes adequate identification for the particular analysis. This concept permits different levels of analysis to be defined. From these levels of analysis, the analyst can select the appropriate level according to both economics and data requirements.

10.8 STANDARDIZED REPORTING FORMAT

A standardized reporting format and statistical evaluation of data have been defined. These incorporate a minimum standard of fiber count reporting, and require that the operator record the classification of each fiber. A computer program is provided which is compatible with the new data reporting technique. For reporting, the user defines the classification categories to be combined in the final result, but the raw data is also presented for re-evaluation of the individual classifications, if necessary.

10.9 INTRODUCTION OF FIBROSITY INDEX

To resolve the cleavage fragment versus fiber controversy in TEM analyses, a "fibrosity index" is defined. On a routine basis it is not possible to determine if a single fiber of a particular aspect ratio is a cleavage fragment or an asbestos fiber. It is possible to distinguish between populations of the two forms on the basis of their aspect ratio distributions. The fibrosity index is a function of the median aspect ratio, and the distribution of aspect ratios higher than the median. A minimum number of about 50 fibers is required in order to define whether or not the population arises from a fibrous mineral.

The question of re-definition of the fiber to one of aspect ratio 10:1 has resolved itself. Even in the case of UICC crocidolite and amosite, 80% of the waterborne fibers have aspect ratios *less than 10:1*; thus, use of the 10:1 definition would be completely unjustified.

10.10 STATISTICS OF FIBER COUNTING

The statistics used to calculate the confidence intervals of measurements have been modified to incorporate logical interpretation of low fiber counts. The 95% confidence interval is calculated on the basis of Poissonian statistics. Where the number of fibers is adequate for calculation of an independent standard deviation on the basis of Gaussian statistics, the corresponding 95% confidence interval is derived and the larger of the two confidence intervals is the one used for reporting. In this way, the more conservative estimate of the analytical precision is used.

10.11 DELETION OF "FIELD OF VIEW" FIBER COUNTING

The alternative "field of view" counting technique for examination of heavily-loaded TEM samples has been deleted. Where this situation exists, the sample should be re-prepared to have a lighter loading. The rationale for this modification was that operators have been found to produce lower fiber counts from heavily-loaded TEM samples, relative to ideally-loaded TEM samples prepared from the same water sample.

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APPENDIX A

TABLES A-1 TO A-7

COLLAPSED MEMBRANE STUDY:
DETAILED ANALYTICAL DATA

TABLE A-1. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE: INITIAL STUDIES

Sample		Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
		Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Nanograms/Liter)	(10^6 Fib/Liter)			x^2	Significance of Uniformity (%)
Nuclepore	1	24.1	19.2 - 29.0	165	0.290	83	10	6.28	50
Controls	2	27.4	21.8 - 33.0	477	0.280	98	10	8.53	25
	3	29.2	24.5 - 33.9	355	0.292	100	10	7.60	50
Collapsed	1	33.5	22.4 - 44.6	392	0.266	126	10	28.4	<0.1
0.1 μm	2	22.5	15.6 - 29.4	192	0.296	76	10	13.74	10
Pore Size	3	22.0	15.7 - 28.3	205	0.271	81	10	12.46	10
Millipore									
Collapsed	1	28.7	21.7 - 35.7	218	0.279	103	10	10.11	25
0.22 μm	2	20.1	13.2 - 27.0	176	0.279	72	10	16.33	5
Pore Size	3	18.5	10.1 - 26.9	194	0.289	64	10	19.75	1
Millipore	4	17.1	9.0 - 25.2	230	0.276	62	10	22.52	0.5
	5	22.5	14.2 - 30.8	190	0.296	76	10	15.84	5
Collapsed	1	13.1	8.0 - 18.2	142	0.272	48	10	12.83	10
0.45 μm	2	16.5	10.1 - 22.9	238	0.275	60	10	15.67	5
Pore Size	3	5.5	1.7 - 9.3	51	0.290	19	10	15.21	5
Millipore									

TABLE A-2. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE

0.1 μm Pore Size Nuclepore Filter Controls

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Nanograms/Liter)	(10^6 Fib/Liter)			x^2	Significance of Uniformity (%)
1	240	160 - 330	1600	1.94	124	7	15.53	1
2	250	180 - 320	1200	2.45	102	5	4.27	25
3	220	160 - 280	1300	2.17	103	6	5.86	25
4	230	160 - 310	2000	2.08	112	6	8.40	10
5	270	180 - 370	1300	2.23	123	6	10.78	5
6	230	160 - 300	1400	2.14	106	6	7.99	10
7	350	310 - 390	2800	3.03	115	4	0.41	90
8	260	120 - 410	1300	2.52	105	5	17.23	0.1
9	270	97 - 450	1600	2.44	112	5	23.99	<0.1
10	310	160 - 470	2100	3.05	103	4	7.17	5

TABLE A-3. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE

0.1 μm Pore Size Millipore Filters

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Nanograms/Liter)	(10^6 Fib/Liter)			x^2	Significance of Uniformity (%)
1	290	170 - 410	1400	2.64	111	5	9.50	2.5
2	350	250 - 450	2300	3.22	108	4	2.76	25
3	290	190 - 390	1200	2.37	123	6	10.46	5
4	340	230 - 450	1600	3.30	102	4	3.28	25
5	300	150 - 450	1300	2.84	105	5	13.84	0.5
6	270	150 - 390	1400	2.65	102	5	10.36	2.5
7	340	190 - 490	1700	3.05	111	4	6.33	5
8	270	230 - 310	1400	2.14	126	6	2.15	75
9	230	190 - 280	1100	2.10	111	6	3.33	50
10	170	150 - 190	980	1.59	107	8	1.55	97.5

TABLE A-4. COLLAPSED MEMBRANE STUDY FOR CHRYSOTILE

0.22 μm Pore Size Millipore Filters

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Nanograms/Liter)	(10^6 Fib/Liter)			X^2	Significance of Uniformity (%)
1	240	180 - 300	1300	2.25	106	6	5.42	25
2	180	140 - 220	1000	1.82	100	7	4.91	50
3	220	140 - 310	1500	1.91	117	7	16.01	1
4*	170	0 - 530	1300	6.35	26	2	0.77	25
5	190	130 - 260	1800	1.90	101	7	12.16	5
6	260	190 - 340	1700	2.52	104	5	4.68	25
7	200	120 - 280	1000	1.92	104	7	15.37	1
8	180	130 - 240	1600	1.63	113	8	12.39	5
9	210	120 - 300	970	1.94	106	7	20.19	0.1
10	180	120 - 230	1000	1.58	112	8	13.77	5

*Unbroken carbon film present on only 2 openings of the 3 specimen grids prepared.

TABLE A-5. COLLAPSED MEMBRANE STUDY FOR CROCIDOLITE

0.1 μm Pore Size Nuclepore Filter Controls

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Micrograms/Liter)	(10^6 Fib/Liter)			x^2	Significance of Uniformity (%)
1	211	168 - 253	63	2.17	97	5	2.07	50
2	259	166 - 352	140	2.35	110	5	7.46	10
3	222	152 - 293	35	2.25	99	5	5.35	25
4	261	238 - 284	43	2.64	99	4	0.23	95
5	226	187 - 265	31	2.24	101	5	1.55	75
6	210	163 - 257	29	2.21	95	5	2.46	50
7	155	110 - 200	129	1.28	121	8	12.8	5
8	256	181 - 331	34	2.49	103	4	2.62	25
9	165	131 - 199	38	1.28	129	8	7.22	25
10	270	216 - 323	45	2.59	104	4	1.28	50

TABLE A-6. COLLAPSED MEMBRANE STUDY FOR CROCIDOLITE

0.1 μm Pore Size Millipore Filters

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Micrograms/Liter)	(10^6 Fib/Liter)			X^2	Significance of Uniformity (%)
1	187	110 - 265	43	1.95	96	6	12.8	2.5
2	211	180 - 242	22	2.17	97	5	1.07	75
3	185	134 - 237	28	1.76	105	6	6.03	25
4	224	167 - 281	26	2.20	102	5	3.40	25
5	218	180 - 255	23	2.20	99	5	1.57	75
6	192	134 - 251	70	1.81	106	6	7.48	10
7	272	187 - 357	107	2.70	101	4	2.87	25
8	262	203 - 322	49	2.10	125	5	3.37	25
9	229	128 - 329	72	2.33	98	7	18.0	0.5
10	310	146 - 475	149	3.07	101	4	8.29	2.5

TABLE A-7. COLLAPSED MEMBRANE STUDY FOR CROCIDOLITE

0.22 μm Pore Size Millipore Filters

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Openings	Grid Distribution	
	Mean (10^6 Fib/Liter)	95% Confidence Interval (10^6 Fib/Liter)	(Micrograms/Liter)	(10^6 Fib/Liter)			X^2	Significance of Uniformity (%)
1	180	147 - 213	32	1.78	101	6	2.61	75
2	196	118 - 275	24	2.18	90	5	7.71	10
3	227	107 - 346	39	2.27	100	5	14.41	0.5
4	181	139 - 224	46	1.93	94	6	3.60	50
5	159	97.6 - 219	18	1.74	91	6	10.1	5
6	223	168 - 279	33	2.09	107	5	3.44	25
7	228	157 - 298	150	1.98	115	5	5.81	10
8	182	128 - 237	84	1.42	128	7	11.6	5
9	208	170 - 246	82	2.12	98	6	2.69	50
10	253	211 - 295	115	2.66	95	5	1.38	75

APPENDIX B

TABLES B-1 TO B-16

REPLICATE ANALYSES OF ENVIRONMENTAL WATER SAMPLES: RESULTS AND STATISTICAL ANALYSES

TABLE B-1. BEAVER BAY, TAP WATER, INITIAL SAMPLE

Sub-Sample Number	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		GRID DISTRIBUTION (TOTAL FIBERS)	
	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	x^2	Significance Level of Uniformity (%)
1	12.1	9.8 - 14.4	20.7	8.05	5.36 - 10.7	1.05	20.2	21.8	29.54	5
2	9.05	6.81 - 11.3	6.77	9.22	6.98 - 11.5	6.67	18.3	13.4	23.54	10
3	7.75	5.95 - 9.55	4.87	8.20	6.27 - 10.1	2.29	16.0	7.16	19.12	10
4	11.4	8.7 - 14.1	8.82	7.02	5.08 - 8.96	7.68	18.4	16.5	38.34	0.5
5	7.85	5.91 - 9.79	5.50	8.64	6.54 - 10.7	3.07	16.5	8.57	29.17	5
6	8.01	5.82 - 10.2	2.59	7.13	4.77 - 9.49	3.89	15.1	6.48	33.99	1
7	5.84	4.21 - 7.47	7.26	7.06	4.99 - 9.13	3.84	12.9	11.1	23.67	10
8	7.89	6.55 - 9.23	7.98	6.95	4.97 - 8.93	2.58	14.8	10.6	13.93	10
9	9.50	6.35 - 12.6	7.74	8.22	6.50 - 9.94	4.32	17.7	12.1	34.62	1
10	9.79	7.05 - 12.5	9.50	5.89	4.58 - 7.20	6.00	15.7	16.5	29.58	5

	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		
ANALYSES OF SUB-SAMPLES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	8.92	1.86	3.47	7.64	0.99	0.98	16.6	2.11	4.46
Mean Mass Concentration (μg/liter)	8.17	4.85	23.5	2.70	2.94	8.64	12.4	4.77	22.7

TABLE B-2. BEAVER BAY, TAP WATER, FINAL SAMPLE

Sub-Sample Number	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		GRID DISTRIBUTION (TOTAL FIBERS)	
	Mean Concentration (10⁶ fib/liter)	95% Confidence Interval (10⁶ fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10⁶ fib/liter)	95% Confidence Interval (10⁶ fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10⁶ fib/liter)	Estimated Mass Concentration (μg/L)	x^2	Significance Level of Uniformity (%)
1	5.79	4.52 - 7.06	11.80	5.70	4.19 - 7.21	0.63	11.5	12.4	18.71	10
2	7.08	5.31 - 8.85	2.96	3.63	2.22 - 5.04	1.23	10.7	4.19	20.80	10
3	5.31	3.89 - 6.73	8.52	3.60	2.35 - 4.85	1.56	8.91	10.1	19.90	10
4	4.88	3.74 - 6.02	3.08	5.32	3.89 - 6.75	10.40	10.2	13.5	12.69	10
5	5.77	3.89 - 7.65	4.44	4.74	3.30 - 6.18	4.74	10.5	9.18	20.37	10
6	5.30	3.66 - 6.94	3.03	5.21	3.95 - 6.47	2.14	10.5	5.17	16.96	10
7	4.94	3.77 - 6.11	2.09	4.39	3.07 - 5.71	2.55	9.33	4.64	24.62	10
8	5.22	3.39 - 7.05	4.46	3.48	1.79 - 5.17	1.53	8.70	5.99	27.66	5
9	3.59	2.17 - 5.01	8.24	5.34	3.87 - 6.81	1.33	8.93	9.57	22.91	10
10	4.07	2.64 - 5.50	2.28	3.89	2.86 - 4.92	0.97	7.96	3.25	22.38	10

	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		
ANALYSES OF SUB-SAMPLES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10⁶ fib/liter)	5.20	0.96	0.92	4.53	0.84	0.71	9.73	1.11	1.24
Mean Mass Concentration (μg/liter)	5.09	3.29	10.8	2.71	2.94	8.64	7.80	3.62	13.1

TABLE B-3. LAKE SUPERIOR WATER

Sub-Sample Number	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		GRID DISTRIBUTION (TOTAL FIBERS)	
	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	x^2	Significance Level of Uniformity (%)
1	1.62	0.59 - 2.65	0.67	3.51	2.15 - 4.87	1.32	5.13	1.97	15.38	10
2	2.07	1.05 - 3.09	13.5	4.13	2.07 - 6.19	2.00	6.20	15.5	23.32	10
3	1.88	0.64 - 3.12	0.81	2.95	1.25 - 4.65	4.78	4.83	5.59	43.05	0.1
4	1.22	0.44 - 2.00	4.22	3.66	2.04 - 5.28	7.52	4.88	11.7	18.85	10
5	1.81	0.63 - 2.99	1.45	3.62	2.31 - 4.93	4.76	5.43	6.21	18.26	10
6	2.04	0.99 - 3.09	2.16	3.40	1.89 - 4.91	1.66	5.44	3.82	18.73	10
7	1.28	0.51 - 2.05	2.20	2.71	0.88 - 4.54	0.40	3.99	2.60	43.05	0.1
8	1.88	0.94 - 2.82	7.67	2.55	0.93 - 4.17	2.86	4.43	10.5	23.81	10
9	1.98	0.84 - 3.12	0.77	4.10	2.33 - 5.87	2.77	6.08	3.54	25.53	10
10	3.27	1.23 - 5.31	3.20	2.86	1.64 - 4.08	0.81	6.13	4.01	23.54	10

ANALYSES OF SUB-SAMPLES	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		
	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	1.90	0.564	0.318	3.35	0.56	0.31	5.25	0.75	0.56
Mean Mass Concentration (μg/liter)	3.66	4.06	16.5	2.89	2.21	4.88	6.55	4.51	20.4

TABLE B-4. DULUTH, RAW WATER

Sub-Sample Number	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		GRID DISTRIBUTION (TOTAL FIBERS)	
	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	95% Confidence Interval (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	Mean Concentration (10^6 fib/liter)	Estimated Mass Concentration (μg/L)	X^2	Significance Level of Uniformity (%)
1	1.65	0.95 - 2.35	1.86	2.55	1.60 - 3.50	2.03	4.20	3.89	25.23	10
2	1.41	0.87 - 1.95	0.75	1.69	0.90 - 2.48	0.41	3.10	1.16	20.66	10
3	1.77	1.02 - 2.52	0.84	1.98	1.28 - 2.68	0.52	3.75	1.36	18.62	10
4	1.64	0.82 - 2.46	1.39	1.78	0.98 - 2.58	0.38	3.42	1.77	32.72	2.5
5	0.68	0.34 - 1.02	0.40	1.16	0.57 - 1.75	1.46	1.84	1.86	16.56	10
6	1.08	0.42 - 1.74	0.77	1.58	0.92 - 2.24	0.60	2.66	1.37	19.69	10
7	1.63	0.80 - 2.46	2.04	1.91	1.17 - 2.65	0.23	3.54	2.27	28.29	5
8	1.57	1.06 - 2.08	0.77	1.71	0.91 - 2.51	1.87	3.28	2.64	16.14	10
9	1.88	1.20 - 2.56	0.83	2.44	1.72 - 3.16	0.68	4.32	1.51	15.60	10
10	1.79	1.17 - 2.41	3.00	2.34	1.47 - 3.21	1.01	4.13	4.01	19.87	10

	AMPHIBOLE - SAED			PROBABLE AMPHIBOLE			TOTAL FIBERS		
ANALYSES OF SUB-SAMPLES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	1.51	0.37	0.13	1.91	0.43	0.18	3.42	0.76	0.58
Mean Mass Concentration (μg/liter)	1.27	0.81	0.65	0.92	0.64	0.41	2.18	1.03	1.06

TABLE B-5. SHERBROOKE, TAP WATER, INITIAL SAMPLE

Sub-Sample Number	Mean Concentration (10^6 fiber/liter)	95% Confidence Interval (10^6 fiber/liter)	Estimated Mass Concentration (μg/L)	Grid Distribution	
				χ^2	Significance Level of Uniformity (%)
1	34.2	22.6 - 45.8	0.22	24.68	0.1
2	51.3	40.5 - 62.1	0.25	14.85	5
3	50.9	37.7 - 64.1	0.31	11.77	10
4	47.9	38.5 - 57.3	0.28	6.98	10
5	50.1	36.7 - 63.5	0.53	13.44	5
6	44.9	32.4 - 57.4	0.27	12.59	5
7	34.0	26.1 - 41.9	0.14	11.84	10
8	41.9	34.3 - 49.5	0.18	5.26	10
9	47.0	32.6 - 61.4	0.32	16.04	1.0
10	40.9	32.5 - 49.3	0.17	6.54	10

OVERALL VALUES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	44.31	6.43	41.36
Mean Mass Concentration (μg/liter)	0.267	0.11	1.2×10^{-2}

TABLE B-6. SHERBROOKE, TAP WATER, FINAL SAMPLE

Sub-Sample Number	Mean Concentration (10^6 fiber/liter)	95% Confidence Interval (10^6 fiber/liter)	Estimated Mass Concentration (μg/L)	Grid Distribution	
				χ^2	Significance Level of Uniformity (%)
1	45.3	40.4 - 50.2	0.26	1.71	10
2	44.7	38.3 - 51.1	0.22	5.68	10
3	41.0	34.3 - 47.7	0.21	6.87	10
4	44.4	34.9 - 53.9	0.27	7.58	10
5	32.3	24.8 - 39.8	0.14	10.81	10
6	51.0	35.8 - 66.2	0.38	17.16	1
7	40.1	33.2 - 47.0	0.20	4.32	10
8	34.7	28.8 - 40.6	0.17	3.80	10
9	36.3	27.1 - 45.5	0.44	8.17	10
10	27.1	20.2 - 34.0	0.22	11.12	10

OVERALL VALUES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	39.69	7.13	50.90
Mean Mass Concentration (μg/liter)	0.25	9.3×10^{-2}	8.65×10^{-3}

TABLE B-7. MAGOG RIVER, SHERBROOKE

Sub-Sample Number	Mean Concentration (10^6 fiber/liter)	95% Confidence Interval (10^6 fiber/liter)	Estimated Mass Concentration (µg/L)	Grid Distribution	
				x^2	Significance Level of Uniformity (%)
1	16.8	11.5 - 22.1	0.13	5.79	10
2	17.3	7.2 - 27.4	0.12	18.56	2.5
3	14.2	9.8 - 18.6	0.045	4.04	10
4	18.6	13.3 - 23.9	0.13	4.85	10
5	18.6	10.5 - 26.7	1.09	11.74	10
6	14.3	10.0 - 18.6	0.38	4.12	10
7	15.5	10.1 - 20.9	0.13	6.20	10
8	16.5	10.1 - 22.9	0.085	8.29	10
9	12.4	7.4 - 17.4	0.070	6.31	10
10	12.4	6.8 - 18.0	0.15	7.99	10

OVERALL VALUES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	15.66	2.29	5.25
Mean Mass Concentration (µg/liter)	0.233	0.315	9.9×10^{-2}

TABLE B-8. SHERBROOKE, RAW WATER

Sub-Sample Number	Mean Concentration (10^6 fiber/liter)	95% Confidence Interval (10^6 fiber/liter)	Estimated Mass Concentration (μg/L)	Grid Distribution	
				χ^2	Significance Level of Uniformity (%)
1	29.9	19.5 - 40.3	0.22	24.68	0.1
2	30.1	23.6 - 36.6	0.25	9.10	10
3	24.9	20.1 - 29.7	0.14	6.18	10
4	23.5	19.0 - 28.0	0.11	5.43	10
5	22.6	15.7 - 29.5	0.18	14.76	5
6	23.2	16.4 - 30.0	0.23	12.72	10
7	27.6	21.0 - 34.2	0.13	10.65	10
8	24.6	17.4 - 31.8	0.13	13.30	10
9	22.8	13.9 - 31.7	0.14	21.39	1.0
10	19.7	14.7 - 24.7	0.15	8.55	10

OVERALL VALUES	Mean ($\bar{x}$)	Standard Deviation (S)	Variance (S^2)
Mean Fiber Concentration (10^6 fib/liter)	24.89	3.35	11.22
Mean Mass Concentration (μg/liter)	0.17	4.9×10^{-2}	2.4×10^{-3}

TABLE B-9. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: BEAVER BAY, TAP WATER, INITIAL SAMPLE

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	9.45	3.84	14.71	11.52	4.16	17.32	2.07	2.01	Marginal
2				10.36	3.58	12.85	0.91	1.75	Yes
3				8.85	3.03	9.19	0.60	1.50	Yes
4				10.82	4.73	22.40	1.37	2.27	Yes
5				9.28	3.83	14.64	0.17	1.86	Yes
6				8.64	3.90	15.21	0.81	1.86	Yes
7				7.41	3.04	9.24	2.04	1.50	No
8				8.67	2.54	6.45	0.78	1.29	Yes
9				10.28	4.33	18.75	0.83	2.09	Yes
10				8.65	3.62	13.10	0.80	1.76	Yes

*Amphibole Random SAED plus Probable Amphibole

TABLE B-10. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: BEAVER BAY, TAP WATER, FINAL SAMPLE

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided *t*-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	5.48	2.47	6.09	6.46	2.50	6.26	0.98	1.21	Yes
2				6.08	2.56	6.56	0.60	1.24	Yes
3				4.93	2.28	5.20	0.55	1.11	Yes
4				5.87	1.99	3.97	0.39	0.98	Yes
5				6.09	2.56	6.54	0.61	1.24	Yes
6				5.94	2.34	5.46	0.46	1.14	Yes
7				5.13	2.57	6.62	0.35	1.24	Yes
8				4.88	2.59	6.73	0.60	1.25	Yes
9				4.85	2.43	5.88	0.63	1.17	Yes
10				4.41	2.36	5.56	1.07	1.15	Yes

*Amphibole Random SAED plus Probable Amphibole

TABLE B-11. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: LAKE SUPERIOR WATER

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	1.91	1.60	2.55	1.89	1.25	1.56	0.02	0.62	Yes
2				2.21	1.68	2.81	0.30	0.81	Yes
3				1.75	1.98	3.91	0.16	0.95	Yes
4				1.81	1.34	1.78	0.10	0.66	Yes
5				1.96	1.37	1.89	0.05	0.67	Yes
6				1.97	1.38	1.90	0.06	0.68	Yes
7				1.32	1.76	3.09	0.59	0.84	Yes
8				1.67	1.54	2.37	0.24	0.75	Yes
9				2.28	1.74	3.04	0.37	0.84	Yes
10				2.23	1.87	3.50	0.32	0.90	Yes

*Amphibole Random SAED plus Probable Amphibole

TABLE B-12. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: DULUTH, RAW WATER

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	2.45	1.69	2.88	3.04	1.95	3.80	0.59	0.94	Yes
2				2.21	1.54	2.37	0.24	0.75	Yes
3				2.70	1.63	2.66	0.25	0.79	Yes
4				2.46	1.96	3.85	0.01	0.94	Yes
5				1.38	1.11	1.22	1.07	0.56	No
6				1.86	1.38	1.90	0.59	0.68	Yes
7				2.48	1.93	3.73	0.03	0.93	Yes
8				2.31	1.41	2.00	0.14	0.70	Yes
9				3.11	1.59	2.53	0.66	0.77	Yes
10				3.01	1.78	3.17	0.56	0.86	Yes

*Amphibole Random SAED plus Probable Amphibole

TABLE B-13. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: SHERBROOKE, TAP WATER, INITIAL SAMPLE

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	16.21	5.42	29.37	12.50	5.93	35.16	3.71	4.32	Yes
2				18.80	5.53	30.58	2.59	4.02	Yes
3				18.25	5.65	31.92	2.04	4.70	Yes
4				17.25	4.04	16.32	1.04	3.44	Yes
5				18.88	6.05	36.60	2.67	5.11	Yes
6				16.63	5.54	30.69	0.42	4.62	Yes
7				12.60	4.11	16.89	3.61	3.06	No
8				16.00	3.48	12.11	0.21	2.99	Yes
9				17.75	6.52	42.51	1.54	5.48	Yes
10				14.88	3.65	13.32	1.33	3.12	Yes

*Chrysotile SAED plus Chrysotile Morphology Only

TABLE B-14. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: SHERBROOKE, TAP WATER, FINAL SAMPLE

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	14.45	4.62	21.34	17.38	2.23	4.97	2.93	1.98	No
2				16.80	3.37	11.36	2.35	2.52	Yes
3				14.50	3.33	11.09	0.05	2.50	Yes
4				16.63	4.25	18.06	2.18	3.58	Yes
5				11.00	3.56	12.67	3.45	2.65	No
6				19.00	6.79	46.10	4.55	5.65	Yes
7				14.75	3.02	9.12	0.30	2.59	Yes
8				13.13	2.66	7.08	1.32	2.31	Yes
9				13.13	4.00	16.00	1.32	3.39	Yes
10				9.50	3.40	11.56	4.95	2.54	No

*Chrysotile SAED plus Chrysotile Morphology Only

TABLE B-15. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: MAGOG RIVER, SHERBROOKE

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided *t*-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	2.88	1.58	2.48	3.20	1.42	2.02	0.32	1.04	Yes
2				3.10	2.54	6.45	0.22	1.82	Yes
3				2.60	1.13	1.28	0.28	0.33	Marginal
4				3.40	1.34	1.80	0.52	0.99	Yes
5				3.40	2.08	4.33	0.52	1.51	Yes
6				2.60	1.09	1.19	0.28	0.81	Yes
7				2.90	1.42	2.02	0.02	1.04	Yes
8				3.10	1.69	2.86	0.22	1.23	Yes
9				2.30	1.29	1.66	0.58	0.95	Yes
10				2.20	1.39	1.93	0.68	1.02	Yes

*Chrysotile SAED plus Chrysotile Morphology Only

TABLE B-16. STATISTICAL ANALYSIS OF REPLICATE SUB-SAMPLING

BULK SAMPLE: SHERBROOKE, RAW WATER

Sub-Sample Number	Number of Fibers per Grid Opening						Two-Sided t-test		Is Sub-Sample Replicate of Mean (at 5% Significance)
	Overall Sample Mean Total Fibers*			Sub-Samples Total Fibers*					
	$\bar{\bar{x}}$	S	S^2	$\bar{x}$	S_n	S_n^2	$\bar{\bar{x}} - \bar{x}$	u	
1	9.29	3.70	13.70	11.50	5.57	31.02	2.21	4.00	Yes
2				11.10	3.36	11.29	1.81	2.45	Yes
3				9.40	2.53	6.40	0.11	1.89	Yes
4				8.30	2.24	5.02	0.99	1.69	Yes
5				8.70	3.74	13.99	0.59	2.73	Yes
6				8.50	3.51	12.32	0.79	2.55	Yes
7				10.50	3.52	12.39	1.21	2.55	Yes
8				8.80	3.60	12.96	0.49	2.61	Yes
9				8.30	4.54	20.61	0.99	3.26	Yes
10				7.60	2.72	7.40	1.69	2.02	Yes

*Chrysotile SAED plus Chrysotile Morphology Only

APPENDIX C

TABLES C-1 TO C-3

CONTAINER AND STORAGE STUDY:
FIBER LENGTH DISTRIBUTIONS

TABLE C-1. FIBER LENGTH DISTRIBUTION: (1st SERIES)
(CUMULATIVE PERCENTAGE NUMBER)

Particle Size Range, µm	Container Material														
	Flint Glass					Polypropylene					Polyethylene				
	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV
0.10 - 0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.15 - 0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.22 - 0.32	1.83	0.00	0.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.95	0.00	0.00
0.32 - 0.47	11.01	13.33	7.34	9.71	8.49	17.76	12.50	10.89	13.86	11.01	18.02	17.00	12.38	7.14	11.71
0.47 - 0.69	41.28	47.62	47.71	46.60	47.17	60.75	41.67	55.45	55.45	57.80	65.77	54.00	57.14	44.90	55.86
0.69 - 1.00	69.72	75.24	73.39	72.82	72.64	81.31	76.39	73.27	78.22	82.57	82.88	74.00	81.90	74.49	84.68
1.00 - 1.50	89.91	89.52	89.91	85.44	92.45	91.59	83.33	91.09	89.11	89.91	93.69	90.00	95.24	89.80	90.99
1.50 - 2.20	96.33	98.10	93.58	97.09	98.11	98.13	91.67	95.05	98.02	96.33	97.30	97.00	97.14	98.98	97.30
2.20 - 3.20	98.17	98.10	97.25	98.06	100.00	98.13	98.61	97.03	99.01	98.17	99.10	99.00	98.10	98.98	99.10
3.20 - 4.70	99.08	98.10	99.08	99.03	100.00	99.07	98.61	98.02	99.01	100.00	99.10	100.00	99.05	100.00	100.00
4.70 - 6.90	100.00	98.10	100.00	100.00	100.00	100.00	98.61	100.00	100.00	100.00	100.00	100.00	99.05	100.00	100.00
6.90 - 10.00	100.00	100.00	100.00	100.00	100.00	100.00	98.61	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
10.00 - 15.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15.00 - 22.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
22.00 - 32.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

TABLE C-2. FIBER LENGTH DISTRIBUTION: (2nd SERIES)

(CUMULATIVE PERCENTAGE NUMBER)

Particle Size Range, µm	Container Material														
	Flint Glass					Polypropylene					Polyethylene				
	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Hand Shake	Routine Prep.	48 hr. Shake	Ozone UV
0.10 - 0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.15 - 0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.22 - 0.32	0.00	0.00	1.08	0.00	0.89	0.00	2.50	0.00	0.00	0.00	1.11	1.75	0.00	3.45	0.00
0.32 - 0.47	16.83	8.45	9.14	15.04	22.32	10.67	37.50	18.35	3.77	15.13	18.89	21.05	20.37	6.90	18.47
0.47 - 0.69	55.45	59.15	54.84	58.41	68.30	54.67	52.50	61.47	43.40	68.07	57.78	64.91	66.67	51.72	67.52
0.69 - 1.00	83.17	81.69	78.49	78.76	87.05	78.67	72.50	85.32	79.25	88.24	80.00	84.21	81.48	86.21	86.62
1.00 - 1.50	93.07	92.96	90.86	96.46	94.64	93.33	85.00	95.41	94.34	95.80	90.00	94.74	93.52	89.66	94.90
1.50 - 2.20	98.02	98.59	97.31	99.12	96.43	97.33	92.50	99.08	96.23	98.32	95.50	98.25	98.15	100.00	97.45
2.20 - 3.20	99.01	100.00	98.39	100.00	97.77	97.33	95.00	99.08	98.11	99.16	100.00	98.25	98.15	100.00	98.73
3.20 - 4.70	99.01	100.00	100.00	100.00	99.11	98.67	95.00	99.08	100.00	100.00	100.00	98.25	99.07	100.00	99.36
4.70 - 6.90	99.01	100.00	100.00	100.00	99.55	98.67	100.00	99.08	100.00	100.00	100.00	100.00	100.00	100.00	99.36
6.90 - 10.00	100.00	100.00	100.00	100.00	99.55	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
10.00 - 15.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15.00 - 22.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
22.00 - 32.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

TABLE C-3. FIBER LENGTH DISTRIBUTION: (3rd SERIES)

(CUMULATIVE PERCENTAGE NUMBER)

Particle Size Range, µm	Container Material														
	Flint Glass					Polypropylene					Polyethylene				
	On Site	Sampling by Pipet*	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Sampling by Pipet*	Routine Prep.	48 hr. Shake	Ozone UV	On Site	Sampling by Pipet*	Routine Prep.	48 hr. Shake	Ozone UV
0.10 - 0.15	0.00	0.00	0.00	Not Applicable	0.00	Not Analyzed	0.00	0.00	Not Applicable	0.00	Not Analyzed	0.00	0.00	Not Applicable	0.00
0.15 - 0.22	0.00	0.00	0.00		0.00		0.00	0.00		0.00		0.00	0.00		0.00
0.22 - 0.32	1.87	1.02	0.00		0.83		0.00	0.00		0.00		0.00	0.00		0.00
0.32 - 0.47	24.30	11.22	9.80		14.17		8.57	15.24		11.72		9.00	13.51		8.00
0.47 - 0.69	66.36	64.29	51.96		58.33		60.00	54.29		54.69		55.00	52.25		52.00
0.69 - 1.00	89.72	87.76	77.45		83.33		77.14	76.19		79.69		84.00	80.18		75.20
1.00 - 1.50	95.33	95.92	87.25		93.33		82.86	87.62		92.19		92.00	92.79		92.00
1.50 - 2.20	98.13	95.92	96.08		98.33		88.57	92.38		98.44		97.00	99.10		96.80
2.20 - 3.20	98.13	96.94	99.02		99.17		97.14	96.19		98.44		99.00	100.00		98.40
3.20 - 4.70	100.00	97.96	100.00		99.17		100.00	99.05		100.00		99.00	100.00		99.20
4.70 - 6.90	100.00	100.00	100.00		99.17		100.00	99.05		100.00		99.00	100.00		100.00
6.90 - 10.00	100.00	100.00	100.00		100.00		100.00	100.00		100.00		99.00	100.00		100.00
10.00 - 15.00	100.00	100.00	100.00		100.00		100.00	100.00		100.00		100.00	100.00		100.00
15.00 - 22.00	100.00	100.00	100.00		100.00		100.00	100.00		100.00		100.00	100.00		100.00
22.00 - 32.00	100.00	100.00	100.00		100.00		100.00	100.00		100.00		100.00	100.00		100.00

*Sub-sample taken from bottle before any shaking.

APPENDIX D

TABLES D-1 AND D-2

CONDENSATION WASHER STUDY: DETAILED ANALYTICAL DATA

TABLE D-1. CONDENSATION WASHER STUDY USING CHRYSOTILE

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Squares	Grid Distribution	
	Mean (10⁶ Fib/Liter)	95% Confidence Interval (10⁶ Fib/Liter)	(Nanograms/Liter)	(10⁶ Fib/Liter)			x^2	Significance of Uniformity (%)
6	298	199 - 397	2750	2.38	125	5	7.18	10
7	235	189 - 281	1180	2.20	107	6	3.16	50
9	268	151 - 384	1200	2.52	106	5	10.1	2.5
10	231	176 - 287	1120	2.18	106	6	4.56	25

TABLE D-2. CONDENSATION WASHER STUDY USING CROCIDOLITE

Sample	Fiber Concentration		Estimated Mass Concentration	Concentration Equivalent to 1 Fiber Detected	No. Fibers Counted	No. Grid Squares	Grid Distribution	
	Mean (10⁶ Fib/Liter)	95% Confidence Interval (10⁶ Fib/Liter)	(Micrograms/Liter)	(10⁶ Fib/Liter)			x^2	Significance of Uniformity (%)
2	226	163 - 289	137	2.31	98	5	3.94	25
3	236	168 - 304	152	2.20	107	5	4.55	25
4	285	216 - 353	20.5	2.66	107	5	3.21	50
5	226	128 - 325	22.2	2.41	94	5	9.60	2.5